

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 June 2002 (20.06.2002)

PCT

(10) International Publication Number
WO 02/48397 A2

(51) International Patent Classification⁷: **C12Q 1/68**,
C12N 9/88, A61K 39/00, 48/00, A61P 11/06, 29/00,
31/00, 37/08

(21) International Application Number: PCT/EP01/14398

(22) International Filing Date: 7 December 2001 (07.12.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/254,782 11 December 2000 (11.12.2000) US

(71) Applicant (for all designated States except US): **BAYER AKTIENGESELLSCHAFT** [DE/DE]; 51368 Leverkusen (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **LIU, Ningshu** [CN/JP]; 4-39-305, Sanjomiyamae-cho, Nara-shi, Nara 630-8121 (JP). **MANABE, Yoshihisa** [JP/JP]; 4-8-19, Suzaku, Nara-shi, Nara 631-0806 (JP).

(74) Common Representative: **BAYER AKTIENGESELLSCHAFT**; 51368 Leverkusen (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report
- entirely in electronic form (except for this front page) and available upon request from the International Bureau

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: REGULATION OF HUMAN ADENYLATE CYCLASE, TYPE VII

(57) Abstract: An adenylate cyclase type VII protein, cDNA, and reagents which regulate human adenylate cyclase type VII can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, diseases that are caused by aberrant activity of this enzyme and diseases whose symptoms can be ameliorated by stimulating or inhibiting the activity of type VII adenylate cyclase. Such diseases include, for example, diseases caused by allergic or inflammatory reaction, such as asthma, allergic rhinitis, atopic dermatitis, food allergy, contact allergy, hives conjunctivitis, vernal catarrh; autoimmune diseases, COPD, inflammatory bowel diseases, rheumatoid arthritis, osteoarthritis, sepsis/septic shock, systemic inflammatory response syndrome, polymyositis, dermatomyositis, polyarthritis nodosa, mixed connective tissue disease, Sjogren's syndrome, gout; and bacterial, fungal, and viral infections, among others.

Rest Available Copy

REGULATION OF HUMAN ADENYLATE CYCLASE, TYPE VII

TECHNICAL FIELD OF THE INVENTION

5 The present invention relates to human adenylate cyclase type VII (hAC7) and the regulation of hAC7.

BACKGROUND OF THE INVENTION

10 The common mechanism for cells to process external stimuli incorporates a receptor at the cell surface to be activated by the stimulus. The activation of the receptor propagates signal to the cell interior and recruits several additional proteins in the process. Typical example of the receptor is a member of G-protein-coupled membrane receptors. The receptors affect the activity of a variety of effectors including ion channels and enzymes, such as adenylyl cyclases, phospholipases, and
15 protein kinases. Each of these effectors includes isoforms with different regulatory properties allowing complex signal integration, and then the signal integration presents the opportunities for the cell to engineer highly specific responses to an external stimulus.

20 The adenylyl cyclase family contains a group of enzymes that synthesize cAMP, a key second messenger involved in signaling pathways governing many cellular processes (e.g. cell growth, development, metabolism and differentiation). To date, nine ACs, were identified principally from rodents. The structure of adenylyl cyclases is well studied and shows a common topology with five domains in
25 sequence: a cytoplasmic N-terminal region; a membrane-anchoring hydrophobic domain (M1) consisting of six transmembrane helices; a large cytoplasmic domain (C1); a second transmembrane helical cluster (M2); and a second cytoplasmic domain (C2), homologous to the first, at the C-terminus. The predicted topology of AC1-AC9 resembles that of the ATP-binding cassette (ABC) membrane transporter
30 such as the P-glycoprotein, although there is no evidence that any mammalian AC functions as a channel or pump to date. All nine AC isoforms contain at least one

site predicted to undergo N-linked glycosylation in M2. Following the demonstration by Tang and Gilman of a recombinant soluble AC entirely lacking M1 and M2, a large body of biochemical and structural evidence has made it clear that the interaction of the homologous C1 and C2 domains lies at the heart of the cyclase catalytic mechanism. It is likely that all isoforms share a common catalytic mechanism that requires dimerization of identical or homologous domains.

Although the nine adenylyl cyclases share common sequences and functional similarities (e.g., all can be activated by the G_{α} proteins), each is under very distinct regulatory mechanisms and expressed in a tissue-specific manner (1-5). Thus, AC3, AC5 and AC6 have been shown to be sensitive to inhibition by G_i protein, AC2, but not AC3 or AC6 can be stimulated by $G_{\beta\gamma}$ subunits. In addition to the direct interaction with subunits of membrane-anchored G proteins, adenylyl cyclases also respond indirectly as a consequence of stimuli-induced alteration of intracellular ionic composition and kinase activity, or both. AC1, AC3 and AC8 are stimulated by Ca^{2+} /calmodulin. AC5 and AC6 are inhibited by low levels of Ca^{2+} . AC2 and AC7 are stimulated by activation of protein kinase C, while G_s -stimulated but not basal activity of AC7 is inhibited by protein kinase C. AC1, AC3, and AC8 with significant expression in the central nervous system, but not other isoforms, demonstrate robust stimulation by Ca^{2+} /CaM. AC5 and AC6 are expressed dominantly in the heart.

Diversity in activation mechanism, and differences in distribution of adenylyl cyclase isoforms, may contribute to tissue specific regulation of cAMP level. The distinct features in structure and biochemical properties strongly suggest that isoform specific modulators can be discovered and are effective in a tissue- and pathology-specific manner.

The nucleotide sequence and the amino acid sequence of human adenylate cyclase VII are available (KIAA0037), but the characteristics of adenylate cyclase type VII has not been studied in detail yet.

SUMMARY OF THE INVENTION

5 It is an object of the present invention to provide reagents and methods for regulating adenylate cyclase type VII. This and other objectives of the invention are provided by one of the embodiments described below.

10 One embodiment of the invention is a method of screening for agents which can regulate the activity of adenylate cyclase type VII. A test compound is contacted with a polypeptide comprising an amino acid sequence which is at least about 70% identical to the amino acid sequence shown in SEQ ID NO: 2. Binding of the test compound to the polypeptide is detected. A test compound which binds to the polypeptide is thereby identified as a potential therapeutic agent for regulating the activity of adenylate cyclase type VII.

15 Another embodiment of the invention is a method of screening for agents which decrease the activity of adenylate cyclase type VII. A test compound is contacted with a polynucleotide encoding a adenylate cyclase polypeptide, wherein the polynucleotide comprises a nucleotide sequence which are at least about 70% identical to the nucleotide sequence shown in SEQ NO:1.

20

Another embodiment of the invention is a method of screening for agents which regulate a biological activity mediated by a adenylate cyclase type VII. A test compound is contacted with a polypeptide comprising an amino acid sequence which is at least about 70% identical to the amino acid sequence shown in SEQ ID NO: 2. A biological activity mediated by the polypeptide is detected. A test compound which decreases the biological activity is thereby identified as a potential therapeutic agent for decreasing the biological activity of the adenylate cyclase type VII. A test compound which increases the biological activity is thereby identified as a potential therapeutic agent for increasing the biological activity of the human adenylate cyclase type VII.

25

30

Yet another embodiment of the invention is a method of screening for agents which regulate an activity of a human adenylate cyclase type VII. A test compound is contacted with a product encoded by a polynucleotide which comprises a nucleotide sequence which is at least 70% identical to the nucleotide sequence shown in SEQ ID NO: 1. Binding of the test compound to the product is detected. A test compound which binds to the product is thereby identified as a potential therapeutic agent for regulating the activity of the human adenylate cyclase type VII.

Even another embodiment of the invention is a method of reducing activity of a human adenylate cyclase type VII. A cell is contacted with a reagent which specifically binds to a product encoded by a polynucleotide comprising a nucleotide sequence which is at least 70% identical to the nucleotide sequence shown in SEQ ID NO: 1. The activity of the human is adenylate cyclase type VII thereby reduced.

Even another embodiment of the invention is a pharmaceutical composition comprising a reagent which specifically binds to a product encoded by a polynucleotide comprising a nucleotide sequence which is at least 70% identical to the nucleotide sequence shown in SEQ ID NO: 1 and a pharmaceutically acceptable carrier.

Another embodiment of the invention is the use of a reagent which specifically binds to a product encoded by a polynucleotide comprising a nucleotide sequence which is at least about 70% identical to the nucleotide sequence shown in SEQ ID NO: 1 in the preparation of a medicament for the treatment of diseases that are caused by aberrant activity of this enzyme and diseases whose symptoms can be ameliorated by stimulating or inhibiting the activity of type VII adenylyl cyclase. Such diseases include, for example, diseases caused by allergic or inflammatory reaction, such as asthma, allergic rhinitis, atopic dermatitis, food allergy, contact allergy, hives conjunctivitis, vernal catarrh; autoimmune diseases, COPD, inflammatory bowel diseases, rheumatoid arthritis, osteoarthritis, sepsis/septic shock, systemic

inflammatory response syndrome, polymyositis, dermatomyositis, polyarthriti nodosa, mixed connective tissue disease, Sjoegren's syndrome, gout; and bacterial, fungal, and viral infections, among others.

- 5 Thus, the invention provides a human adenylate cyclase type VII, which can be regulated to provide therapeutic effects.

BRIEF DESCRIPTION OF THE DRAWING

- 10 Fig. 1 shows the DNA sequence encoding a human adenylate cyclase type VII polypeptide.

Fig. 2 shows the amino acid sequence deduced from the DNA sequence of Fig. 1.

Fig. 3 shows a PCR primer used to amplify DNA complementary to human adenylate cyclase type VII mRNA in various tissues.

- 15 Fig. 4 shows a PCR primer used to amplify DNA complementary to human adenylate cyclase type VII mRNA in various tissues.

Fig. 5 shows a PCR primer used to amplify DNA complementary to human adenylate cyclase type VII mRNA in various immune cells.

- 20 Fig. 6 shows a PCR primer used to amplify DNA complementary to human adenylate cyclase type VII mRNA in various immune cells.

Fig. 7 shows the expression profiles of hAC isoforms.

Fig. 8 shows the expression profiles of hAC 7 in immune cells.

Fig. 9 shows the DNA sequence including the sequence encoding a human adenylate cyclase type VII polypeptide and untranslated region.

25

DETAILED DESCRIPTION OF THE INVENTION

- It is a discovery of the present invention that human adenylate cyclase type VII (AC7) can be regulated to control diseases that are caused by aberrant activity of this enzyme and diseases whose symptoms can be ameliorated by stimulating or
30 inhibiting the activity of type VII adenylyl cyclase.

More specifically, the fact that specific PDE (phosphodiesterase, a down-stream enzyme regulates cAMP level through converting cAMP into the corresponding 5-monophosphate inactive counterparts) namely PDE4 inhibitors become promising agents for the treatment of asthma or other inflammatory or allergic diseases supports the idea that the specific modulators of adenylyl cyclase may also have the same or synergetic roles in the therapy for asthma or other allergic or inflammatory diseases. Further, present inventors' discovery that AC7 is expressed dominantly in immune system and lung tissue in human indicates that AC7 acts as a central enzyme that regulates the production of cAMP to modulate immune responses.

The expression of hAC7 in lung and trachea also shows that hAC7 activity potentially plays a role in the respiratory system. It has been shown that pulmonary endothelium forms a semi-selective barrier that regulates fluid balance and leukocyte trafficking. During the course of lung inflammation, neurohumoral mediators and oxidants act on endothelial cell to induce intercellular gaps permissive for transudation of proteinaceous fluid from blood into the interstitium. Cytosolic Ca^{2+} concentration and cAMP are suggested to be two important signals that dictate cell-cell apposition involved in such an inflammation course: the elevated concentration of endothelial cell $[\text{Ca}^{2+}]_i$ decreases cAMP to facilitate intercellular gap formation. (Moore, TM, et al, 1998); and increased $[\text{Ca}^{2+}]_i$ promotes disruption of the macrovascular endothelial cell barrier, while increased cAMP enhances endothelial barrier function. Further, cAMP functions as a getting element on the chemoattractant-induced rho-dependent signaling pathway leading to leukocyte integrin activation and adhesion. cAMP, acting through protein kinase A, inhibits chemoattractant-triggered integrin-dependent leukocyte adhesion. cAMP- induced inhibition of rho activation may be responsible for the anti-inflammatory activity of cAMP elevating agonists and drugs. (Laudanna C, et al, 1997). Drugs that modulate the activity of hAC7 are expected to have anti-inflammatory effects by inhibiting chemotaxis and intercellular gap formation.

Therefore, AC 7 can be regulated to control diseases such as caused by allergic or inflammatory reaction, such as asthma, allergic rhinitis, atopic dermatitis, food allergy, contact allergy, hives conjunctivitis, vernal catarrh; autoimmune diseases, COPD, inflammatory bowel diseases, rheumatoid arthritis, osteoarthritis, sepsis/septic shock, systemic inflammatory response syndrome, polymyositis, dermatomyositis, polyarthritis nodosa, mixed connective tissue disease, Sjogren's syndrome, gout; and bacterial, fungal, and viral infections, among others.

Polypeptides

10

Adenylate cyclase type VII polypeptides according to the present invention comprises at least 14, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, or 250 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO: 2 or a biologically active variant thereof, as defined below. An adenylate cyclase type VII polypeptide of the invention therefore can be a portion of an adenylate cyclase type VII protein, a full-length adenylate cyclase type VII protein, or a fusion protein comprising all or a portion of an adenylate cyclase type VII protein.

Biologically Active Variants

Adenylate cyclase type VII polypeptide variants are biologically active, *i.e.*, retain a catalytic activity to convert ATP to adenosine 3', 5'-cyclic monophosphate (cAMP). Adenylate cyclase are known for a number of activities which can be monitored *in vitro*. For example, adenylate cyclase activity is assayed indirectly by measuring the synthesis of radioactively labeled cAMP from a substrate as described in Anal. Biochem., 58, 541 (1974) and Adv. Cyclic Nucleotide Res., 10, 35 (1979).

Preferably, naturally or non-naturally occurring adenylate cyclase type VII polypeptide variants have amino acid sequences which are at least about 70, preferably about 75, 90, 96, or 98% identical to the amino acid sequence shown in

SEQ ID NO: 2 or a fragment thereof. Percent identity between a putative adenylate cyclase type VII polypeptide variant and an amino acid sequence of SEQ ID NO: 2 is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48:603 (1986), and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (ibid.). Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative variant. The FASTA algorithm is described by Pearson and Lipman, Proc. Nat'l Acad. Sci. USA 85:2444(1988), and by Pearson, Meth. Enzymol. 183:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (e.g. SEQ ID NO: 2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, J. Mol. Biol. 48:444 (1970); Sellers, SIAM J. Appl. Math. 26:787 (1974)), which allows for amino acid insertions and deletions. Preferred parameters for FASTA analysis are: ktup=1, gapopeningpenalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file

5 ("SMATRIX"), as explained in Appendix 2 of Pearson, Meth. Enzymol. 183:63 (1990). FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as default.

10 Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

15 Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of an adenylate cyclase type VII polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active adenylate
20 cyclase type VII polypeptide can readily be determined by assaying for the efficiency of converting ATP into cAMP.

Fusion Proteins

25 Fusion proteins are useful for generating antibodies against adenylate cyclase type VII polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins, which interact with portions of an adenylate cyclase type VII polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the
30 yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

An adenylate cyclase type VII polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 14, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, or
5 250 contiguous amino acids of SEQ ID NO:2 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length adenylate cyclase type VII.

The second polypeptide segment can be a full-length protein or a protein fragment.
10 Proteins commonly used in fusion protein construction include beta-galactosidase, beta-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including
15 histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between
20 the adenylate cyclase type VII polypeptide-encoding sequence and the heterologous protein sequence so that the adenylate cyclase type VII polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a
25 fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from SEQ ID NO: 1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the
30 DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation

(Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

5

Identification of Species Homologs

Species homologs of human adenylate cyclase type VII polypeptide can be obtained using adenylate cyclase type VII polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of adenylate cyclase type VII polypeptide, and expressing the cDNAs as is known in the art.

10

Polynucleotides

15

An adenylate cyclase type VII-encoding polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for an adenylate cyclase type VII polypeptide. A coding sequence for human adenylate cyclase type VII is shown in SEQ ID NO: 1.

20

Degenerate nucleotide sequences encoding human adenylate cyclase type VII polypeptides, as well as homologous nucleotide sequences which are at least about 70, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NO: 1 are adenylate cyclase type VII polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affinity gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of adenylate cyclase type VII polynucleotides which encode biologically active adenylate cyclase type VII polypeptides also are adenylate cyclase type VII polynucleotides.

25

30

Identification of Polynucleotide Variants and Homologs

5 Variants and homologs of the adenylate cyclase type VII polynucleotides described above also are adenylate cyclase type VII polynucleotides. Typically, homologous adenylate cyclase type VII polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known adenylate cyclase type VII polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, 10 pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50 °C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

15 Species homologs of the adenylate cyclase type VII polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of adenylate cyclase type VII polynucleotides can be identified, for example, 20 by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol.* 81, 123 (1973). Variants of human adenylate cyclase type VII polynucleotides or adenylate cyclase type VII polynucleotides of other species can therefore be identified by hybridizing a putative homologous adenylate 25 cyclase type VII polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO:1 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is 30 calculated.

Nucleotide sequences which hybridize to adenylate cyclase type VII polynucleotides or their complements following stringent hybridization and/or wash conditions also are adenylate cyclase type VII polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*,
5 MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between an adenylate
10 cyclase type VII polynucleotide having a nucleotide sequence shown in SEQ ID NO:1 or the complement thereof and a polynucleotide sequence which is at least about 73, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and
15 McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* 48, 1390 (1962):

$$T_m = 81.5\text{ }^{\circ}\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l$$
, where
 l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65 oC, or 50%
20 formamide, 4X SSC at 42 oC, or 0.5X SSC, 0.1% SDS at 65 oC. Highly stringent wash conditions include, for example, 0.2X SSC at 65 oC.

Preparation of Polynucleotides

25 A naturally occurring adenylate cyclase type VII polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for
30 isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated adenylate cyclase type

VII polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises adenylate cyclase type VII nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

5

Adenylate cyclase type VII cDNA molecules can be made with standard molecular biology techniques, using adenylate cyclase type VII mRNA as a template. Adenylate cyclase type VII cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

10

15

Alternatively, synthetic chemistry techniques can be used to synthesize adenylate cyclase type VII polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode an adenylate cyclase type VII polypeptide having, for example, an amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof.

20

Extending Polynucleotides

25

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic.* 2, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

30

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res.* 16, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72 °C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic.* 1, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res.* 19, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser
5 activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (e.g. GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable
10 for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining Polypeptides

15 Adenylate cyclase type VII polypeptides can be obtained, for example, by purification from human cells, by expression of adenylate cyclase type VII polynucleotides, or by direct chemical synthesis.

Protein Purification

20 Adenylate cyclase type VII polypeptides can be purified from any human cell which expresses the enzyme, including host cells which have been transfected with adenylate cyclase type VII expression constructs. A particular good source of adenylate cyclase type VII is peripheral blood leukocyte cells. A purified adenylate
25 cyclase type VII polypeptide is separated from other compounds which normally associate with the adenylate cyclase type VII polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and
30 preparative gel electrophoresis. The high salt washes are likely to be enriched for adenylate cyclase enzyme. A preparation of purified adenylate cyclase type VII

polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

5 Expression of Polynucleotides

To express an adenylate cyclase type VII polynucleotide, the polynucleotide can be inserted into an expression vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods that are well
10 known to those skilled in the art can be used to construct expression vectors containing sequences encoding adenylate cyclase type VII polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY,
15 John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding an adenylate cyclase type VII polypeptide. These include, but
20 are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial
25 expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with
30 host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including

- 18 -

constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding an adenylate cyclase type VII polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the adenylate cyclase type VII polypeptide. For example, when a large quantity of an adenylate cyclase type VII polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the adenylate cyclase type VII polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem.* 264, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol.* 153, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding adenylate cyclase type VII polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J.* 6, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J.* 3, 1671-1680, 1984; Broglie *et al.*, *Science* 224, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ.* 17, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (e.g., Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express an adenylate cyclase type VII polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding adenylate cyclase type VII polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of adenylate cyclase type VII polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda* cells or

Trichoplusia larvae in which adenylate cyclase type VII polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci.* 91, 3224-3227, 1994).

Mammalian Expression Systems

5

A number of viral-based expression systems can be used to express adenylate cyclase type VII polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding adenylate cyclase type VII polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing an adenylate cyclase type VII polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci.* 81, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

15

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (e.g., liposomes, polycationic amino polymers, or vesicles).

20

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding adenylate cyclase type VII polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding an adenylate cyclase type VII polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of

25

30

various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ.* 20, 125-162, 1994).

5

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed adenylate cyclase type VII polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express adenylate cyclase type VII polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced adenylate cyclase type VII sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell* 11, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell* 22, 817-23, 1980) genes which can be employed in *tk* or *aprf* cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci.* 77, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol.* 150, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci.* 85, 8047-51, 1988). Visible markers such as anthocyanins, β -glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol.* 55, 121-131, 1995).

Detecting Expression

Although the presence of marker gene expression suggests that the adenylate cyclase type VII polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding an adenylate cyclase type VII polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode an adenylate cyclase type VII polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding an adenylate cyclase type VII polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the adenylate cyclase type VII polynucleotide.

Alternatively, host cells which contain an adenylate cyclase type VII polynucleotide and which express an adenylate cyclase type VII polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding an adenylate cyclase type VII polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding an adenylate cyclase type VII polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding an adenylate cyclase type VII polypeptide to detect transformants which contain an adenylate cyclase type VII polynucleotide.

A variety of protocols for detecting and measuring the expression of an adenylate cyclase type VII polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on an adenylate cyclase type VII polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med.* 158, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding adenylate cyclase type VII polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled

- 24 -

nucleotide. Alternatively, sequences encoding an adenylate cyclase type VII polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding an adenylate cyclase type VII polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode adenylate cyclase type VII polypeptides can be designed to contain signal sequences which direct secretion of soluble adenylate cyclase type VII polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound adenylate cyclase type VII polypeptide.

As discussed above, other constructions can be used to join a sequence encoding an adenylate cyclase type VII polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system

(Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the adenylate cyclase type VII polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing an adenylate cyclase type VII polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif.* 3, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the adenylate cyclase type VII polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol.* 12, 441-453, 1993.

Chemical Synthesis

Sequences encoding an adenylate cyclase type VII polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al.* *Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, an adenylate cyclase type VII polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc.* 85, 2149-2154, 1963; Roberge *et al.*, *Science* 269, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of adenylate cyclase type VII polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, *PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES*, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic adenylate cyclase type VII polypeptide can be confirmed

by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the adenylate cyclase type VII polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce adenylate cyclase type VII polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter adenylate cyclase type VII polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of an adenylate cyclase type VII polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab,

F(ab=)₂, and Fv, which are capable of binding an epitope of an adenylate cyclase type VII polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

5

An antibody which specifically binds to an epitope of an adenylate cyclase type VII polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

15

Typically, an antibody which specifically binds to an adenylate cyclase type VII polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to adenylate cyclase type VII polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate an adenylate cyclase type VII polypeptide from solution.

20

Adenylate cyclase type VII polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, an adenylate cyclase type VII polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions,

25

30

keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

5 Monoclonal antibodies which specifically bind to an adenylate cyclase type VII polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature* 256, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods* 81, 31-42, 1985; Cote *et al.*, *Proc. Natl.*
10 *Acad. Sci.* 80, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol.* 62, 109-120, 1984).

In addition, techniques developed for the production of chimeric antibodies, the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*,
15 *Proc. Natl. Acad. Sci.* 81, 6851-6855, 1984; Neuberger *et al.*, *Nature* 312, 604-608, 1984; Takeda *et al.*, *Nature* 314, 452-454, 1985). Monoclonal and other antibodies also can be humanized, to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or
20 may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in
25 GB2188638B. Antibodies which specifically bind to an adenylate cyclase type VII polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can
30 be adapted using methods known in the art to produce single chain antibodies which specifically bind to adenylate cyclase type VII polypeptides. Antibodies with related

specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton, *Proc. Natl. Acad. Sci.* 88, 11120-23, 1991).

5 Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev.* 5, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol.* 15,
10 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem.* 269, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using
15 standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer* 61, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth.* 165, 81-91).

20 Antibodies which specifically bind to adenylate cyclase type VII polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci.* 86, 3833-3837, 1989;
25 Winter *et al.*, *Nature* 349, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and
30 which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which an adenylate cyclase type VII polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of adenylate cyclase type VII gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such as alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. See Brown, *Meth. Mol. Biol.* 20, 1-8, 1994; Sonveaux, *Meth. Mol. Biol.* 26, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev.* 90, 543-583, 1990.

Modifications of adenylate cyclase type VII gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the adenylate cyclase type VII gene. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the

start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of an adenylate cyclase type VII polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to an adenylate cyclase type VII polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent adenylate cyclase type VII nucleotides, can provide sufficient targeting specificity for adenylate cyclase type VII mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular adenylate cyclase type VII polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to an adenylate cyclase type VII polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate

group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. See, e.g., Agrawal *et al.*, *Trends Biotechnol.* 10, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev.* 90, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett.* 215, 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. See, e.g., Cech, *Science* 236, 1532-1539; 1987; Cech, *Ann. Rev. Biochem.* 59, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol.* 2, 605-609; 1992, Couture & Stinchcomb, *Trends Genet.* 12, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (e.g., Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of an adenylate cyclase type VII polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the adenylate cyclase type VII polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (see Haseloff *et al.* *Nature* 334, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within an adenylate cyclase type VII RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate adenylate cyclase type VII RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease adenylate cyclase type VII expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Screening Methods

The invention provides assays for screening test compounds which bind to or modulate the activity of an adenylate cyclase type VII polypeptide or an adenylate cyclase type VII polynucleotide. A test compound preferably binds to an adenylate cyclase type VII polypeptide or polynucleotide. More preferably, a test compound decreases or increases the ability of human adenylate cyclase type VII to convert ATP to cAMP or adenylate cyclase type VII activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacological agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. See Lam, *Anticancer Drug Des.* 12, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (see, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90, 6909, 1993; Erb *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 91, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem.* 37, 2678, 1994; Cho *et al.*, *Science* 261, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.*

33, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2061; Gallop *et al.*, *J. Med. Chem.* 37, 1233, 1994). Libraries of compounds can be presented in solution (see, e.g., Houghten, *BioTechniques* 13, 412-421, 1992), or on beads (Lam, *Nature* 354, 82-84, 1991), chips (Fodor, *Nature* 364, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89, 1865-1869, 1992), or phage (Scott & Smith, *Science* 249, 386-390, 1990; Devlin, *Science* 249, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci.* 97, 6378-6382, 1990; Felici, *J. Mol. Biol.* 222, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

10 High Throughput Screening

Test compounds can be screened for the ability to bind to adenylate cyclase type VII polypeptides or polynucleotides or to affect adenylate cyclase type VII activity or adenylate cyclase type VII gene expression using high throughput screening. Using
15 high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 μ l. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate
20 readers are commercially available to fit the 96-well format.

Alternatively, free format assays, or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by
25 Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 19, 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the
30 active compounds cause the cells to change colors.

- 36 -

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

10

Yet another example is described by Salmon *et al.*, *Molecular Diversity* 2, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

15

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

20

Binding Assays

25

For binding assays, the test compound is preferably a small molecule which binds to and occupies, for example, the ATP/GTP binding site of the enzyme or the active site of the adenylate cyclase type VII polypeptide, such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules.

30

In binding assays, either the test compound or the adenylate cyclase type VII polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic,

chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the adenylate cyclase type VII polypeptide can then be accomplished, for example, by direct counting of radio-emission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to an adenylate cyclase type VII polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with an adenylate cyclase type VII polypeptide. A microphysiometer (*e.g.*, CytosensorTM) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and an adenylate cyclase type VII polypeptide (McConnell *et al.*, *Science* 257, 1906-1912, 1992).

Determining the ability of a test compound to bind to an adenylate cyclase type VII polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem.* 63, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol.* 5, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcoreTM). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, an adenylate cyclase type VII polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell* 72, 223-232, 1993; Madura *et al.*, *J. Biol. Chem.* 268, 12046-12054, 1993; Bartel *et al.*, *BioTechniques* 14, 920-924, 1993; Iwabuchi *et al.*, *Oncogene* 8, 1693-1696, 1993; and Brent W094/10300), to identify other

proteins which bind to or interact with the adenylate cyclase type VII polypeptide and modulate its activity.

5 The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding an adenylate cyclase type VII polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or
10 "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a
15 transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the adenylate cyclase type VII polypeptide.

20 It may be desirable to immobilize either the adenylate cyclase type VII polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the adenylate cyclase type VII polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid
25 supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the adenylate cyclase type VII polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages,
30 passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test

compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to an adenylate cyclase type VII polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the adenylate cyclase type VII polypeptide is a fusion protein comprising a domain that allows the adenylate cyclase type VII polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed adenylate cyclase type VII polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either an adenylate cyclase type VII polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated adenylate cyclase type VII polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to an adenylate cyclase type VII polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the ATP/GTP binding site or the active site of the adenylate cyclase type

VII polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

5 Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the adenylate cyclase type VII polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the adenylate cyclase type VII polypeptide, and SDS gel electrophoresis under non-reducing conditions.

10 Screening for test compounds which bind to an adenylate cyclase type VII polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises an adenylate cyclase type VII polypeptide or polynucleotide can be used in a cell-based assay system. An adenylate cyclase type VII polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to an adenylate cyclase type VII polypeptide or polynucleotide is determined as described above.

Enzyme Assays

20 Test compounds can be tested for the ability to increase or decrease adenylate cyclase type VII activity. Adenylate cyclase activity is assayed indirectly by measuring the synthesis of labeled cAMP from the substrate labeled ATP as described by Y. Salomon et al., as disclosed in Anal. Biochem., 58, 541 (1974) and Adv. Cyclic Nucleotide Res., 10, 35 (1979).

25 Enzyme assays can be carried out after contacting either a purified adenylate cyclase type VII polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound which decreases the activities of an adenylate cyclase type VII polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for decreasing adenylate

30

cyclase type VII activity. A test compound which increases an activity of a human adenylate cyclase type VII polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for increasing human adenylate cyclase type VII activity.

5

Gene Expression

In another embodiment, test compounds which increase or decrease adenylate cyclase type VII gene expression are identified. An adenylate cyclase type VII polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the adenylate cyclase type VII polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of adenylate cyclase type VII mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of an adenylate cyclase type VII polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into an adenylate cyclase type VII polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses an adenylate cyclase type VII polynucleotide can be used in a cell-based assay system. The adenylate cyclase type VII polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, an adenylate cyclase type VII polypeptide, adenylate cyclase type VII polynucleotide, ribozymes or antisense oligonucleotides, antibodies which specifically bind to an adenylate cyclase type VII polypeptide, or mimetics, agonists, antagonists, or inhibitors of an adenylate cyclase type VII polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for

oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

5 Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn,
10 wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

15 Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to
20 the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as
25 glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

30

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated

condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

5

The modulation of adenylate cyclase type VII activity is useful for treating diseases that are caused by aberrant activity of this enzyme and diseases whose symptoms can be ameliorated by stimulating or inhibiting the activity of type VII adenyl cyclase. Such diseases include, for example, diseases caused by allergic or inflammatory
10 reaction, such as asthma, allergic rhinitis, atopic dermatitis, food allergy, contact allergy, hives conjunctivitis, vernal catarrh; autoimmune diseases, COPD, inflammatory bowel diseases, rheumatoid arthritis, osteoarthritis, sepsis/septic shock, systemic inflammatory response syndrome, polymyositis, dermatomyositis, polyarthritis nodosa, mixed connective tissue disease, Sjogren's syndrome, gout;
15 and bacterial, fungal, and viral infections, among others.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For
20 example, an agent identified as described herein (e.g., a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or an adenylate cyclase type VII polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model
25 to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects adenylate cyclase type VII activity can be administered to a
30 human cell, either *in vitro* or *in vivo*, to reduce adenylate cyclase type VII activity. The reagent preferably binds to an expression product of a human adenylate cyclase

type VII gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of

targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

5 Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μ g to about 10 μ g of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μ g to about 5 μ g of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μ g of polynucleotides is combined
10 with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al.* *Trends in Biotechnol.* 11, 202-05 (1993);
15 Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem.* 263, 621-24 (1988); Wu *et al.*, *J. Biol. Chem.* 269, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem.* 266, 338-42 (1991).

20 Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases adenylate cyclase type VII activity
25 relative to the adenylate cyclase type VII activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The
30 animal model also can be used to determine the appropriate concentration range and

route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

5 Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50} .

10 Pharmaceutical compositions, which exhibit large therapeutic indices, are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage
15 form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect.
20 Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on
25 the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available
30 to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of

polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

5 If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, gene gun, and DEAE- or calcium
10 phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 g to about 50 g/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration
15 of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense
20 oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of an adenylate cyclase type VII gene or the
25 activity of an adenylate cyclase type VII polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of an adenylate cyclase type VII gene or the activity of an adenylate cyclase type VII polypeptide can be assessed using methods well known in the art,
30 such as hybridization of nucleotide probes to adenylate cyclase type VII-specific

- 50 -

mRNA, quantitative RT-PCR, immunologic detection of an adenylate cyclase type VII polypeptide, or measurement of adenylate cyclase type VII activity.

5 In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described
10 above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses,
15 rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

Human adenylate cyclase type VII also can be used in diagnostic assays for detecting
20 diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences which encode the enzyme. For example, differences can be determined between the cDNA or genomic sequence encoding adenylate cyclase type VII in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted
25 individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments
30 can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing

primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

5

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g., Myers et al., Science 230, 1242, 1985*). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g., Cotton et al., Proc. Natl. Acad. Sci. USA 85, 4397-4401, 1985*). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

10

15

20

25

Altered levels of an adenylate cyclase type VII also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

30

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following

- 52 -

specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1*Tissue Expression of adenylate cyclase type VII mRNA*

Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of
5 RNA from different human tissues was performed to investigate the tissue expression
of adenylate cyclase mRNA. 100 µg of total RNA from various tissues (Human
Total RNA Panel Stanford G3, Clontech Laboratories, Palo Alto, CA, USA) was
used as a template to synthesize first-strand cDNA using the SUPERSCRIPT™
First-Strand Synthesis System for RT-PCR (Life Technologies, Rockville, MD,
10 USA). 10 ng of the first-strand cDNA was then used as template in a polymerase
chain reaction to test for the presence of the adenylate cyclase type VII mRNA
transcript. The polymerase chain reaction was performed in a LightCycler (Roche
Molecular Biochemicals, Indianapolis, IN, USA), in the presence of the DNA-
binding fluorescent dye SYBR Green I which binds to the minor groove of the DNA
15 double helix, produced only when double-stranded DNA is successfully synthesized
in the reaction, and upon binding, emits light that can be quantitatively measured by
the LightCycler machine. The polymerase chain reaction was carried out using
oligonucleotide primers. The primers used to amplify DNA complementary to AC7
mRNA were primer 1 (SEQ ID NO:3) and primer 2 (SEQ ID NO:4). The
20 measurements of the intensity of emitted light were taken following each cycle of the
reaction when the reaction had reached a temperature of 86 degrees C. Intensities of
emitted light were converted into copy numbers of the gene transcript per nanogram
of template cDNA by comparison with simultaneously reacted standards of known
concentration.

25

To correct for differences in mRNA transcription levels per cell in the various tissue
types, a normalization procedure was performed using calculated expression levels in
the various tissues of five different housekeeping genes: glyceraldehyde-3-
phosphatase (G3PHD), hypoxanthine guanine phosphoribosyl transferase (HPRT),
30 beta-actin, porphobilinogen deaminase (PBGD), and beta-2-microglobulin. Except
for the use of a slightly different set of housekeeping genes, the normalization

procedures was essentially the same as that described in the RNA Master Blot User Manual, Appendix C (Clontech Laboratories, Palo Alto, CA, USA).

5 The expression of mRNA of hAC7 was seen in immune related cells such as thymus and PBL and lung tissue as shown in FIG. 1.

EXAMPLE 2

Expression of recombinant human adenylate cyclase type VII

10 To produce large quantities of human adenylate cyclase type VII polypeptides in yeast, the *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used. The human adenylate cyclase type VII encoding DNA sequence is the nucleotide sequence shown in SEQ ID NO: 1 or SEQ ID NO:7. Before insertion into
15 vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag, and a termination codon. Restriction enzyme recognition sequences for cleavage by restriction endonucleases are added at both termini. After digestion of the multiple cloning site of pPICZ B with the
20 corresponding restriction enzymes, the modified human adenylate cyclase type VII polypeptide-encoding DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, and expression is driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast. The yeast is cultivated under usual conditions in shake flasks, and the recombinantly produced protein is isolated from the culture by affinity chromatography (Ni-NTA-
25 Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the human adenylate cyclase type VII polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human adenylate cyclase type VII polypeptide is obtained.

30

EXAMPLE 3*Identification of test compounds that bind to adenylate cyclase type VII polypeptides*

Purified adenylate cyclase type VII polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96- well
5 microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Adenylate cyclase type VII polypeptides comprise the amino acid sequence shown in SEQ ID NO:2. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour.
10 Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to an adenylate cyclase type VII polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which
15 increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to an adenylate cyclase type VII polypeptide.

EXAMPLE 4

20 *Identification of a test compound which modulates adenylate cyclase type VII gene expression*

A test compound is administered to a culture of human cells transfected with an adenylate cyclase type VII expression construct and incubated at 37 °C for 10 to 45
25 minutes. A culture of the same type of cells which have not been transfected is incubated for the same time without the test compound to provide a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem.* 18, 5294-99 (1979). Northern blots are prepared using 20 to 30 µg total RNA and
30 hybridized with a ³²P-labeled adenylate cyclase type VII-specific probe at 65 °C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides

selected from the complement of SEQ ID NO: 1. A test compound which decreases the adenylate cyclase type VII-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of adenylate cyclase type VII gene expression.

5

EXAMPLE 5

Treatment of a patient with a reagent which specifically binds to an adenylate cyclase human gene mRNA

10 Synthesis of an antisense oligonucleotide comprising at least 11 contiguous nucleotides selected from the complement of SEQ ID NO:1 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoramidite procedure (Uhlmann *et al.*, *Chem. Rev.* 90, 534-83, 1990). Following assembly and deprotection, the oligonucleotide is twice ethanol-precipitated, dried, and suspended
15 in phosphate-buffered saline (PBS) at the desired concentration. Purity of the oligonucleotide is tested by capillary gel electrophoreses and ion exchange HPLC. The endotoxin level in the oligonucleotide preparation is determined using the *Limulus* Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.)* 105, 361-362, 1953).

20

An aqueous composition containing the antisense oligonucleotides at a concentration of 0.1-100 μ M is administered directly to a patient having by injection. The severity of the patient is decreased.

25

EXAMPLE 6

Expression of human adenylate cyclase type VII in various immune cells

Cells. Eosinophils(purity > 99%) were isolated with Mono-Poly resolving medium (Dainippon Pharmaceutical Co. Ltd.) followed with lysis of erythrocytes and
30 negative selection with CD16, CD4, CD8, CD19 using autoMACS. Neutrophils were

prepared from PBMC by positive selection with anti-CD16 antibody. Purity was checked by FACS analysis. Both eosinophils and neutrophils have >99% purity.

RNA isolation and RT-PCR. Total RNAs were prepared using TRIzol™ Reagent (GIBCO BRL) or purchased from Clontech. First-strand cDNA synthesis was carried out with SuperScript™ first-strand synthesis system (GIBCO BRL). PCR reactions were performed with either GeneAmp PCR system 9700 or LightCycler (Roche). The following primers are used:

AC7- L: 5'-tcagcgtcgcctcagggcacgag-3' (SEQ ID NO:5)

AC7-R: 5'-gaggccctggaggatggtgcag-3' (SEQ ID NO:6)

Expression of AC7 in immune cells is shown in FIG. 8

References

(Sunahara, R. K., Dessauer, C. W., and Gilman, A. G. (1996) Annu. Rev. Pharmacol. Toxicol. 36, 461-480)

(Xia, Z. G., and Storm, S. R. (1997) Curr. Opin. Neurobiol. 7, 391-396)

(Hanoune, J., Pouille, Y, Tzavara, E., Shen, T. S., Lipskaya, L., Miyamoto, N., Suzuki, Y., and Defer, N. (1997) Mol. Cell. Endocrinol. 128, 179-194)

(Cooper, D. M. F. (ed) (1998) Adv. Second Messenger Phosphoprotein Res. 32)

(Tang, WJ and Hurley, JH (1998) Ml. Pharmacol. 54, 231-240)

(Morre, TM, Chetham, PM, Kelly, JJ., and Stevens, T., Am. L. Physiol. 275:L203-L222, 1998)

(Laudanna, C., Campbell, JJ., and Butcher, EC., 1997, J. Biol. Chem., 272:24141-24144).

EXAMPLE 7*In vivo testing of compounds/target validation for asthma treatment*

1. Tests for activity of T cells

5

Costimulatory molecules-cytokines, cytokine receptors, signalling molecules, any molecule involved in T cell activation

Mouse anti-CD3 induced cytokine production model

10

BALB/c mice were injected with a single intravenous injection of 10 µg of 145-2C11 (purified hamster anti-mouse CD3 ε monoclonal antibodies, PHARMINGEN). Compound was administered intraperitoneally 60 min prior to the anti-CD3 mAb injection. Blood was collected 90 min after the antibody injection. Serum was obtained by centrifugation at 3000 r.p.m. for 10 min. IL-2 and IL-4 levels in the serum was determined by an ELISA.

15

2. Tests for activity of B cells

20

B cell receptor, signalling molecules, any molecule involved in B cell activation/Ig class switching

Mouse anti-IgD induced IgE production model

25

BALB/c mice were injected intravenously with 0.8 mg of purified goat anti-mouse IgD antibody or PBS (defined as day 0). Compound was administered intraperitoneally from day 0 to day 6. On day 7 blood was collected and serum was obtained by centrifugation at 3000 r.p.m. for 10 min. Serum total levels of IgE were determined by YAMASA's ELISA kit and their Ig subtypes were done by an Ig ELISA KIT (Rougier Bio-tech's, Montreal, Canada).

30

3. Tests for activity of monocytes/macrophages, signalling molecules, Transcription factors

Mouse LPS-induced TNF- α production model

5

BALB/c mice were injected intraperitoneally with LPS (200 μ g/mouse). Compound was administered intraperitoneally 1 hr before the LPS injection. Blood was collected at 90 min post-LPS injection and plasma was obtained. TNF- α concentration in the sample was determined using an ELISA kit.

10

4. Tests eosinophil activation

Eotaxin-eotaxin receptor (GPCR)

15

Signalling molecules, Cytoskeletal molecules, adhesion molecules

Mouse eotaxin-induced eosinophilia model

20

BALB/c mice were injected intradermally with a 2.5 ml of air on days -6 and -3 to prepare airpouch. On day 0 compound was administered intraperitoneally 60 min before eotaxin injection (3 μ g/mouse, i.d.). IL-5 (300 ng/mouse) was injected intravenously 30 min before the eotaxin injection. After 4 hr of the eotaxin injection leukocytes in exudate was collected and the number of total cells was counted. The differential cell counts in the exudate were performed by staining with May-Grunwald Gimsa solution.

25

5. Tests activation of Th2 cells

Molecules involved in antigen presentation, costimulatory molecules, signaling molecules, transcription factors

30

Mouse D10 cell transfer model

- 60 -

D10.G4.1 cells (1×10^7 cells/mouse) containing 2 mg of conalbumin in saline was administered i.v. to AKR mice. After 6 hr blood was collected and serum was obtained by centrifugation at 3000 r.p.m. for 10min. IL-4 and IL-5 level in serum were determined by ELISA kits. Compound was administered intraperitoneally at -4 and +1 hr after these cells injection.

6. Passive cutaneous anaphylaxis (PCA) test in rats

6 Weeks old male Wistar rats are sensitized intradermally (i.d.) on their shaved backs with 50 μ l of 0.1 μ g/ml mouse anti-DNP IgE monoclonal antibody (SPE-7) under a light anesthesia. After 24 hours, the rats are challenged intravenously with 1 ml of saline containing 0.6 mg DNP-BSA (30) (LSL CO., LTD) and 0.005 g of Evans blue. Compounds are injected intraperitoneally (i.p.) 0.5 hr prior to antigen injection. Rats without the sensitization, challenge, and compound treatment are used for a blank (control) and rats with sensitization, challenge and vehicle treatment are used to determine a value without inhibition. Thirty min after the challenge, the rats are killed, and the skin of the back is removed. Evans blue dye in the skin is extracted in formamide overnight at 63°C. Then an absorbance at 620 nm is measured to obtain the optical density of the leaked dye.

Percent inhibition of PCA with a compound is calculated as follows:

% inhibition = $\{(\text{mean vehicle value} - \text{sample value}) / (\text{mean vehicle value} - \text{mean control value})\} \times 100$

7. Anaphylactic bronchoconstriction in rats

6 Weeks old male Wistar rats are sensitized intravenously (i.v.) with 10 μ g mouse anti-DNP IgE, SPE-7, and 1 days later, the rats are challenged intravenously with 0.3 ml of saline containing 1.5 mg DNP-BSA (30) under anesthesia with urethan (1000

- 61 -

mg/kg, i.p.) and gallamine (50 mg/kg, i.v.). The trachea is cannulated for artificial respiration (2 ml / stroke, 70 strokes / min). Pulmonary inflation pressure (PIP) is recorded through a side-arm of cannula connected to pressure transducer. Change in PIP reflects change of both resistance and compliance of the lungs. To evaluate the

5 drugs, each drug is given i.v. 5 min before challenge.

CLAIMS

1. A method of screening for agents which decrease the activity of adenylyl cyclase type VII, comprising the steps of:
- 5 contacting a test compound with any adenylyl cyclase type VII polypeptide encoded by any polynucleotide selected from the group consisting of:
- 10 a) a polynucleotide encoding an adenylyl cyclase type VII polypeptide comprising an amino acid sequence which is at least about 70% identical to the amino acid sequence shown in SEQ ID NO: 2, or the amino acid sequence shown in SEQ ID NO: 2;
- b) a polynucleotide comprising the sequence of SEQ ID NO: 1;
- c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and/or (b) and encodes an adenylyl cyclase type VII polypeptide;
- 15 d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes an adenylyl cyclase type VII polypeptide; and
- e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes an adenylyl cyclase type VII polypeptide;
- 20 and detecting binding of the test compound to the adenylyl cyclase type VII polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of adenylyl cyclase type VII.
- 25
2. A method of screening for agents which regulate the activity of adenylyl cyclase type VII, comprising the steps of:
- 30 contacting a test compound with any adenylyl cyclase type VII polypeptide encoded by any polynucleotide selected from the group consisting of:

- 5
- a) a polynucleotide encoding an adenylate cyclase type VII polypeptide comprising an amino acid sequence which is at least about 70% identical to the amino acid sequence shown in SEQ ID NO: 2, or the amino acid sequence shown in SEQ ID NO: 2;
- b) a polynucleotide comprising the sequence of SEQ ID NO: 1;
- c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and/or (b) and encodes an adenylate cyclase type VII polypeptide;
- 10 d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes an adenylate cyclase type VII polypeptide; and
- e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes an adenylate cyclase type VII polypeptide;
- 15

and detecting adenyl cyclase type VII activity of the polypeptide, wherein a test compound which increases the adenylate cyclase type VII activity is identified as a potential therapeutic agent for increasing the activity of adenylate cyclase type VII, and wherein a test compound which decreases the adenylate cyclase type VII activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of adenyl cyclase type VII.

20

- 25 3. A method of screening for agents which decrease the activity of adenyl cyclase type VII, comprising the steps of:

contacting a test compound with any adenylate cyclase type VII polynucleotide selected from the group consisting of:

30

- 5
- a) a polynucleotide encoding an adenylate cyclase type VII polypeptide comprising an amino acid sequence which at least about 70% identical to the amino acid sequence shown in SEQ ID NO: 2, or the amino acid sequence shown in SEQ ID NO: 2;
- b) a polynucleotide comprising the sequence of SEQ ID NO: 1;
- c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and/or (b) and encodes an adenylate cyclase type VII polypeptide;
- 10 d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes an adenylate cyclase type VII polypeptide; and
- e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and
- 15 encodes an adenylate cyclase type VII polypeptide;

and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of adenyl cyclase type VII.

20

4. A method of reducing the activity of adenyl cyclase type VII, comprising the steps of:
- contacting a cell with a reagent which specifically binds to any polynucleotide selected from the group consisting of:
- 25 a) a polynucleotide encoding an adenylate cyclase type VII polypeptide comprising an amino acid sequence which at least about 70% identical to the amino acid sequence shown in SEQ ID NO: 2, or the amino acid sequence shown in SEQ ID NO: 2;
- b) a polynucleotide comprising the sequence of SEQ ID NO: 1;

- 5
- c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and/or (b) and encodes an adenylate cyclase type VII polypeptide;
 - d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes an adenylate cyclase type VII polypeptide; and
 - e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes an adenylate cyclase type VII polypeptide;
- 10
- or
- 15
- any polypeptide encoded by a polynucleotide selected from the group consisting of:
- a) a polynucleotide encoding an adenylate cyclase type VII polypeptide comprising an amino acid sequence which at least about 70% identical to the amino acid sequence shown in SEQ ID NO: 2, or the amino acid sequence shown in SEQ ID NO: 2;
 - 20 b) a polynucleotide comprising the sequence of SEQ ID NO: 1;
 - c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and/or (b) and encodes an adenylate cyclase type VII polypeptide;
 - d) 25 a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes an adenylate cyclase type VII polypeptide; and
 - e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes an adenylate cyclase type VII polypeptide,
- 30

whereby the activity of adenylate cyclase type VII is reduced.

5. A reagent that modulates the activity of adenyl cyclase type VII polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 1 to 3.
6. A pharmaceutical composition, comprising:
the reagent of claim 5 and a pharmaceutically acceptable carrier.
7. Use of the reagent of claim 5 in the preparation of a medicament for modulating the activity of adenyl cyclase type VII in a disease.
8. Use of claim 7 wherein the disease is an infectious disease, asthma or an allergic or inflammatory disease.
9. A method of screening for agents which can regulate the activity of adenylate cyclase type VII protein, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid sequence which is at least about 70% identical to the amino acid sequence shown in SEQ ID NO: 2; or the sequence shown in SEQ ID NO: 2;
and detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the adenylate cyclase type VII protein.
10. The method of claim 9 wherein the step of contacting is in a cell.
11. The method of claim 9 wherein the cell is *in vitro*.
12. The method of claim 9 wherein the step of contacting is in a cell-free system.

13. The method of claim 9 wherein the polypeptide comprises a detectable label.
14. The method of claim 9 wherein the test compound comprises a detectable label.
15. The method of claim 9 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.
16. The method of claim 9 wherein the polypeptide is bound to a solid support.
17. The method of claim 9 wherein the test compound is bound to a solid support.
18. A method of screening for agents which regulate the activity of adenylate cyclase type VII protein, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid sequence which is at least about 70% identical to the amino acid sequence shown in SEQ ID NO: 2; or the sequence shown in SEQ ID NO: 2; and
detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human adenylate cyclase type VII protein, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human adenylate cyclase type VII protein.
19. The method of claim 18 wherein the step of contacting is in a cell.
20. The method of claim 18 wherein the cell is *in vitro*.

21. The method of claim 18 wherein the step of contacting is in a cell-free system.
22. A method of screening for agents which regulate adenylate cyclase type VII protein, comprising the steps of:
5 contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO:1; and
detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for
10 regulating the activity of human adenylate cyclase type VII protein.
23. The method of claim 22 wherein the product is a polypeptide.
24. The method of claim 22 wherein the product is RNA.
15
25. A method of reducing activity of a human adenylate cyclase type VII protein, comprising the step of:
contacting a cell with a reagent which specifically binds to a product encoded
by a polynucleotide comprising the nucleotide sequence shown in SEQ ID
20 NO:1, whereby the activity of a human adenylate cyclase type VII protein is reduced.
26. The method of claim 25 wherein the product is a polypeptide.
- 25 27. The method of claim 26 wherein the reagent is an antibody.
28. The method of claim 25 wherein the product is RNA.
29. The method of claim 28 wherein the reagent is an antisense oligonucleotide.
30

30. The method of claim 29 wherein the reagent is a ribozyme.
31. The method of claim 25 wherein the cell is *in vitro*.
- 5 32. The method of claim 25 wherein the cell is *in vivo*.
33. A pharmaceutical composition, comprising:
a reagent which specifically binds to a polypeptide comprising the amino acid
sequence shown in SEQ ID NO:2; and
10 a pharmaceutically acceptable carrier.
34. The pharmaceutical composition of claim 33 wherein the reagent is an
antibody.
- 15 35. A pharmaceutical composition, comprising:
a reagent which specifically binds to a product of a polynucleotide comprising
the nucleotide sequence shown in SEQ ID NO:1; and
a pharmaceutically acceptable carrier.
- 20 36. The pharmaceutical composition of claim 35 wherein the reagent is a
ribozyme.
37. The pharmaceutical composition of claim 35 wherein the reagent is an
antisense oligonucleotide.
- 25 38. The pharmaceutical composition of claim 35 wherein the reagent is an
antibody.

39. A method of treating adenyl cyclase tpye VII dysfunction related disease, wherein the disease is selected from an infectious disease, asthma, or an allergic or inflammatory disease comprising the step of:
administering to a patient in need thereof a therapeutically effective dose of a
5 reagent that regulates the function of human adenyl cyclase tpye VII protein, whereby symptoms of the adenyl cyclase tpye VII dysfunction related disease are ameliorated.
40. The method of claim 39 wherein the reagent is identified by the method of
10 claim 9.
41. The method of claim 39 wherein the reagent is identified by the method of claim 18.
42. The method of claim 39 wherein the reagent is identified by the method of
15 claim 22.

FIG. 1

atgccagccaaggggcgctacttcctcaacgagggcgaggagggccctgaccaa
gatgcgctctacgagaagtaccagctcaccagccagcatggggccgctgctgctc
acgctcctgctgggtggccgcccactgcctgcggtggccctcatcatcattgccttc
agccaggggggacccctccagacaccaggccattctgggcatggcggttcctgggtg
ctggcggtggtttgcggccctctctgtgctgatgtacgtcgagtgctctcctgcgg
cgctgggtcagggcccttggcgctgctcacctgggcctgcttgggtggcgctggggc
tatgtgctgggtgttcgacgcatggacaaaaggcggcctgtgctggtgggagcaggtg
cccttcttctgctgttcattgtcttcgtgggtgtacacactactgcccttcagcatg
cggggcgctgtcgccgttggggcgctctccactgcctcccacctcctgggtgctc
gggttctttgatgggaggttcacgacacccagtggtccgggtggggctgcagctg
ctggccaacgcagtcattctcctgtgtgggaacctgacaggcgccctccacaag
caccaaatgcaggatgcgtcccgggacctcttcacctacactgtgaagtgcac
cagatccgcccgaagctgcgcatcgagaagcgccagcaggagaacctgctgctg
tcagtgttccggcccacatctccatgggcatgaagctggccatcatcgaacgg
ctcaaggagcatgggtgaccgtcgctgcatgcctgacaacaacttccacagcctc
tacgtcaagaggcaccagaatgtcagcatcctctatgcggaacatcgtgggcttc
acgcagctggccagcgactgttctcccaaggagctgggtgggtggtgctgaatgag
ctctttggcaagttcgaccagatcgccaaggccaacgagtgcatgcgaatcaag
atcctcggcgactgctactactgtgtatcgggcctgcccgtgtcgctgcctacc
cacgcccggaactgcgtgaagatggggctggacatgtgccaggccatcaagcag
gtgcgggaggccacgggctggacatcaacatgcgtgtgggcatacactcgggg
aatgtgctgtgcggggtcatcgggctgcgcaagtggcagtatgacgtgtgggtcc
cacgacgtgtccctggccaaccggatggaggcagccggagtaccgggcccgggtg
cacatcacggaggccacgctaaagcacctggacaaggcgtacgaggtggaggat
gggcacgggagcagcgggacccctacctcaaggagatgaacatccgcacctac
ctgggtcatcgacccccggagccagcagccacccccgcccagccaacacctcccc
aggcccaagggggagcgcgccctgaagatgcgggcgtcagtgcgcatgaccgg
tacctcgagtcctggggggcgggcacggccctttgcacatctcaaccacgtgag
agcgtgagcagtggtgagacccacgtccccaacgggcgaggccctaagagcgtt
ccccagcgccaccgcccggacccccagacagaagcatgtccccaaggggcggtcg
gaggatgactcgtacgatgacgagatgctgtcagccattgaggggctcagctcc
acgaggccctgctgctccaagtccgatgacttctacacctttgggtccatcttc
ctggagaagggtttgagcgcgagtaccgcctggcacccatcccccgggcccg
cacgactttgcctgcgcccagcctgatcttcgtctgcatcctgctcgtccatgtc
ctgctcatgccaggacggcggcactgggtgtgtccttcgggctgggtggcctgt
gtactggggctgggtgctgggcctgtgctttgccaccaagtctcagaggtgctgc
ccagctcgggggagcgtctgcactatctctgagaggggtggagacacagccctg
ctgaggctgacctggccgtcctgacctcggcagcctgctcactgtggccatc
atcaacctgcccctgatgcctttccaagttccagagctgcctgttggcaatgag
acaggcctactggccgagcagcaagacaagagccctgtgtgagccctccc
tactacacctgcagctgtgtcctgggcttcacgcctgctcggtcttcctgagg
atgagcctggagccaaaggttgtgctgctgacagtgggccctgggtggcctacctg

- 2/9 -

FIG. 1 (continued)

gtgctcttcaacctctccccatgctggcagtgaggactgctgcgcccaaggcctg
ggcaacctcaccaagcccaacggcaccaccagtgccacccctagctgttctctgg
aaggacctgaagaccatgaccaatttctacctggctctgttctacatcacctg
cttacctctccagacagattgactattactgccgcttggactgcctatggaag
aagaagttcaagaaggagcacgaggagtttgagaccatggagaacgtgaaccgc
cttcttctggagaacgtcctgccagcccacgtggctgcccactttatcggtgac
aagttaaacgaggactggtaccatcagtcctatgactgcgtctgtgtcatgttt
gcctccgtgccggacttcaaagtgttctacacagagtgcgatgtcaacaaagaa
gggctggagtgcctacgcctgctcaatgagatcattgccgacttcgacgagctc
ctactgaagcccaagttcagcggcggtggagaagatcaagaccatcggcagcacg
tacatggcagctgcaggggtcagcgtcgctcagggcacgagaaccaggagctg
gagcggcagcatgcccacattggtgtcatggtggagttcagcatcgccctgatg
agtaagctggacggcatcaacaggcactccttcaactccttccgcctccgcgtc
ggcataaaccatgggcctgtgattgctggagtgattggggcccgaaaacctcag
tatgacatctggggaaacactgtcaatgtggccagccgaatggaaagcactgga
gaacttgggaaaatccaggttaccgaggagacctgcaccatcctccagggcctc
gggtactcttgtgaatgccgtggcctgatcaacgtcaaaggcaaaggcgagctg
aggacttactttgtctgtacggacactgccaagtttcaggggctggggctgaac
tga

FIG. 2

MPAKGRYFLNEGEEGPDQDALYKEYQLTSQHGPLLLTLLLVAATACVALIIIAF
SQGDPSRHQAILGMAFLVLAVFAALSVMYVECLLRRLRALALLTWACLVALG
YVLVFDATKAACAWEQVPFFLFIVFVYTLFPFSMRGAVAVGAVSTASHLLVL
GSLMGGFTTPSVRVGLQLLANAVIFLCGNLTGAFHKHQMQDASRDLFYTVKCI
QIRRLRIEKROQENLLLSVLPAHISMGMKLAIIERLKEHGDRCMPDNNFHSL
YVKRHQNVSIYADIVGFTQLASDCSPKELVVVLNELFGKFDQIAKANECMRIK
ILGDCYYCVSGLPVSLPHTARNVCVKMLDMCQAIKQVREATGVDINMRVGIHSG
NVLCGVIGLRKWQYDVWSHDVSLANRMEAAGVPGRVHITEATLKHLDKAYEVED
GHGQQRDPYLKEMNIRTYLVIDPRSQQPPPPSQHLPRPKGDAALKMRASVRMTR
YLESWGAARPF AHLNHRESVSSGETHV PNGRRPKSVPQRHRRTPD RSMSPKGRS
EDDSYDDEMLSAIEGLSSTRPCCSKSDDFYTFGSI FLEKGFEREYRLAPIPRAR
HDFACASLIFVCILLVHVLLMPRTAALGVSFGLVACVLGLVLGLCFATKFSRCC
PARGTLCTISERVETQPLLRLTLAVLTIGSLLTVAIINLPLMPFQVPELPVGNE
TGLLAASSKTRALCEPLPYTTCSCVLGFIACSVFLRMSLEPKVVLLTVALVAYL
VLFNLSPCWQWDCCGQGLGNLT KPNGTTS GTPSCSWKDLKTM TNFYLVLFYITL
LTL SRQIDYYCRLDCLWKKKFKKEHEEFETMENVNRLLLE NVLPAHVA AHFIGD
KL NEDWYHQSYDCVCVMFASVPDFKVFYTECDVNKEGLECLRLLNEI IADFDEL
LLKPKFSGVEKIKTIGSTYMAAAGLSVASGHENQELERQHAHIGVMVEFSIALM
SKLDGINRHSFNSFRLRVGINHGPVIAGVIGARKPOYDIWGNTVNVASRMESTG
ELGKIQVTEETCTILQGLGYSCECRGLINVKGKGELRTYFVCTDTAKFQGLGLN

- 3/9 -

FIG. 3

ccggacccccagacagaagcatg

FIG. 4

ggatgcagacgaagatcaggctg

FIG. 5

AC7- L: 5'-tcagcgtcgcctcagggcacgag-3'

FIG. 6

AC7-R: 5'-gagggccctggaggatggtgcag-3'

- 4/9 -

FIG. 7

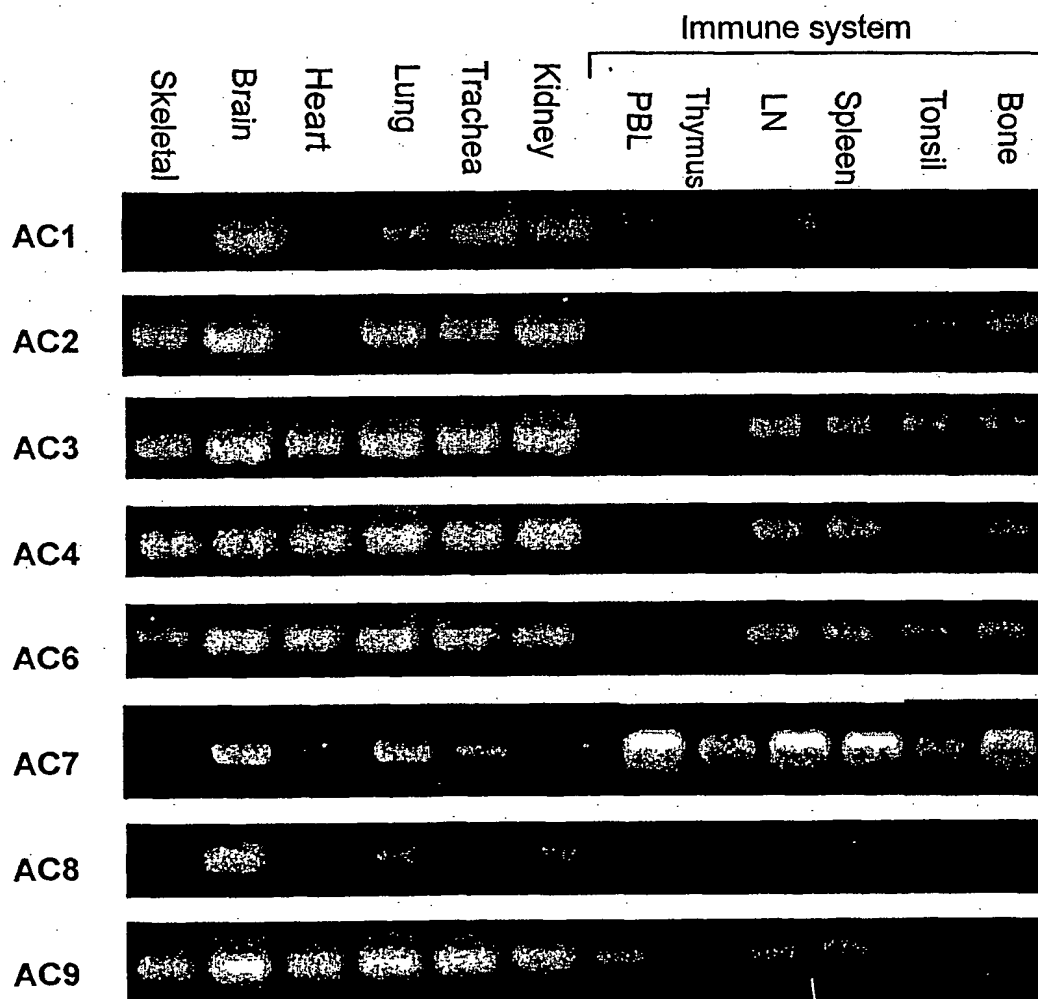


FIG. 8

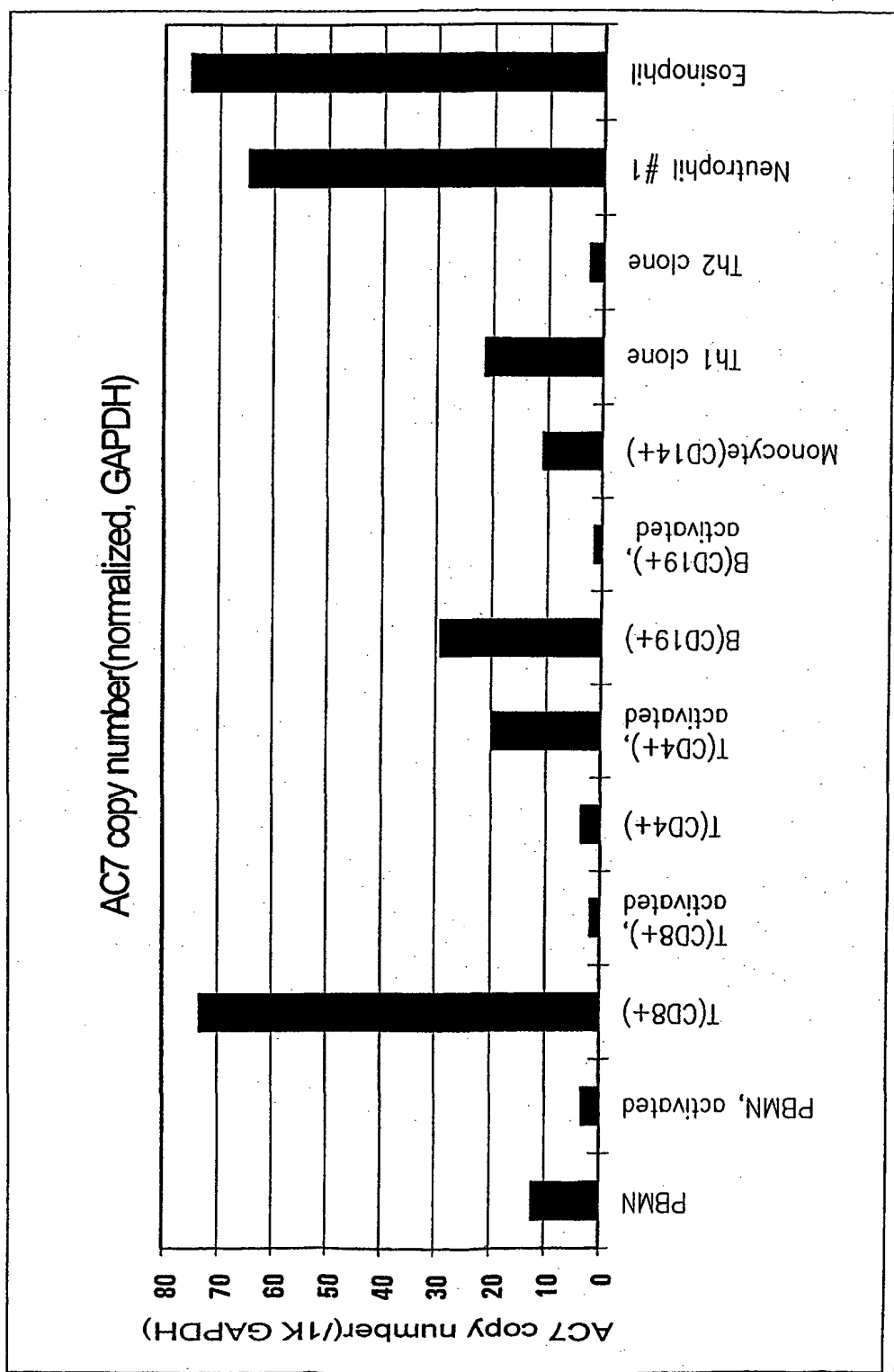


FIG. 9

tgaggaaactgcgtgtggagtcagcccagtcctggatgcacaggaggatgctggcg
gcacagtgagtgaggcctggtgccagagctgtgcggaccccttggttgccatgg
agcagcaggcccagaggccctctccccagccctgcttgccctgcctcggagagga
cagaggcctaggcccacgggggaggggtgttggcagacagatgccctccaggccc
tggggcctccttaacggcccttaacgacacgcgtgccaaaggggtggaggatgcc
agccaaggggcgctacttcctcaacgagggcgaggagggccctgaccaagatgc
gctctacgagaagtaccagctcaccagccagcatggggccgctgctgctcacgct
cctgctggtggccgcccactgcctgctggccctcatcatcattgccttcagcca
gggggacccctccagacaccaggccattctgggcattggcgcttcctgggtgctggc
gggtgtttgcggccctctctgtgtgatgtacgtcgagtgctcctgcggcgctg
gctcagggccttggcgctgctcacctgggcctgcttgggtggcgctgggctatgt
gctggtgttcgacgcatggacaaaggcggcctgtgcgtgggagcaggtgccctt
cttcctgttcattgtcttcgtggtgtacacactactgcccttcagcatgcgggg
cgctgtcgccgcttggggccgctctccactgcctcccacctcctgggtgctcggttc
tttgatgggaggcttcacgacaccagtggtccgggtggggctgcagctgctggc
caacgcagtcattctcctgtgtgggaacctgacaggcgccttccacaagcacca
aatgcaggatgcgtcccgggacctcttcacctacactgtgaagtgcattccagat
ccgcccgaagctgcgcattcgagaagcgccagcaggagaacctgctgctgtcagt
gcttcgggcccacatctccatgggcatgaagctggccatcatcgaacggctcaa
ggagcatgggtgaccgtcgctgcatgcctgacaacaacttccacagcctctacgt
caagaggcaccagaattgtcagcatcctctatgcggacatcggtgggcttcacgca
gctggccagcgactgttctcccaaggagctgggtgggtggtgctgaatgagctctt
tggcaagttcgaccagatcgccaaaggccaaacgagtgcatgcgaatcaagatcct
cggcgactgctactactgtgtatcgggcctgcccgtgtcgctgcctacccacgc
ccggaactgcgtgaagatggggctggacatgtgccaggccatcaagcaggtgcg
ggaggccacgggctggacatcaacatgcgtgtgggcatacactcgggggaatgt
gctgtgcggggctcatcgggctgcgcaagtggcagtatgacgtgtggtcccacga
cgtgtccctggccaaccggatggaggcagccggagtaccgggcccgggtgcacat
cacggaggccacgctaaagcacctggacaaggcgtacgaggtggaggatgggca
cgggcagcagcgggacccctacctcaaggagatgaacatccgcacctacctgggt
catcgacccccggagccagcagccaccccgccagccaacacctccccaggcc
caagggggagcgcggccctgaagatgcgggctcagtgccgcatgacccggtaacct
cgagtcctggggggcgggcacggccctttgcacatctcaaccaccgtgagagcgt
gagcagtggtgagacccacgtccccaaacgggcgaggcctaagagcgttccccca
gcgccaccgcccggaccccagacagaagcatgtcccccaaggggctgggagga
tgactcgtacgatgacgagatgctgtcagccattgaggggctcagctccacgag
gccctgctgctccaagtccgatgacttctacacctttgggtccatcttcctgga
gaagggctttgagcgcgagtagccgctggcaccatccccgggcccggccacga
ctttgcctgcgccagcctgatcttcgtctgcatcctgctcgtccatgtcctgct
catgcccaggacggcggcactgggtgtgtccttcgggctgggtggcctgtgtact
ggggctgggtgctgggcctgtgctttggccaccaagtctcagaggtgctggccagc
tcgggggacgctctgcactatctctgagaggggtggagacacagccctgctgag
gctgaccctggcgctcctgacctcggcagcctgctcactgtggccatcatcaa
cctgcccctgatgcctttccaagtccagagctgcctgttggcaatgagacagg
cctactggccgagcagcaagacaagagccctgtgtgagccctcccgtacta
cacctgcagctgtgtcctgggcttcattgcctgctcggtcttcctgaggatgag
cctggagccaaagggttggtgctgctgacagtgggccctgggtggcctacctgggtgct
cttcaacctctccccatgctggcagtgaggactgctgcggccaaaggcctgggcaa
cctcaccaagcccaacggcaccaccagtgggcaccctagctgttcctggaagga

FIG. 9 (continued)

cctgaagaccatgaccaatttctacctggctcctgttctacatcacccctgcttac
actctccagacagattgactattactgccgcttgactgcctatggaagaagaa
gttcaagaaggagcacgaggagtgttgagaccatggagaacgtgaaccgccttct
tctggagaacgtcctgccagcccacgtggctgcccactttatcgggtgacaagtt
aaacgaggactggtaccatcagtcctatgactgcgctctgtgtcatgtttgcct
ccgtgccggacttcaaagtgttctacacagagtgcgatgtcaacaaagaagggc
tggagtgcctacgcctgctcaatgagatcattgccgacttcgacgagctcctac
tgaagcccaagttcagcggcgtggagaagatcaagaccatcggcagcacgtaca
tggcagctgcagggctcagcgtcgcctcagggcacgagaaccaggagctggagc
ggcagcatgcccacattggtgtcatggtggagttcagcatcgccctgatgagta
agctggacggcatcaacaggcactccttcaactccttccgcctccgcgtcggca
taaaccatgggcctgtgattgctggagtgattggggcccgaaaacctcagtatg
acatctggggaaacactgtcaatgtggccagccgaatggaaagcactggagaac
ttgggaaaatccagggtaccgaggagacctgcaccatcctccagggcctcgggt
actcttgtgaatgccgtggcctgatcaacgtcaaaggcaaaggcgagctgagga
cttactttgtctgtacggacactgccaaagtttcaggggctggggctgaactgag
ggctcctgctggattccgaaaaggccgggaagccagctctccttccctgaagcaa
gccaggagaagactctccgccccacgccaatcccaaaggcatgcagatggctg
tgcattgttggttctttggacctgcactggaggatttctcagacacatgcacca
gattctggctcgaagcagccactgagccataatgcgcaggggaggccagaagct
ctgtgcctggctctgtaacagtttccaggccagctggagaatgttactggttcg
gggctgacttttgagatctttgttccctgaggtgccaggcaggcaactttagcac
atgatgaaaacagacttccacctcagtggtcctgtgggcacgcacaagtgaggtc
tgtttttctagacaccaaggggggagtaagctgagctgtctagcacggattggag
actccctctccctgggtgggcctggcaatgacagcatttctcacagaggcatttct
ggtaaatgaagctgaaaggggtgttttacatctgtaaacgggttcaaacaggta
gagagaaaaacaccacaattaacactgttactttttgccttgctctggcatgttt
gttttaaatgaatacattaatgggggtttttatccttttgaaatgacttttcagac
actagacataaatctcttccctccagtgtatgctctgcctttttaaccactgac
atgtaaggaggactactgtctagcatcagcttatgggggtcagctggctgtgggg
atagagtccctgaggaatgtgggtcacagcaagaaggcggggagcagcagagcctt
gcctttgaatgaggcagcttgtgaggcaagcattctggagagaggtgctttgaa
agtaagggtgcggcctttcacctcttcccttgattactcacacatctttgcgttct
cccctgcgctccttcaactgtatcttacttttcttaccagaaaggaatggagtc
tgttttagagacaacttggaacacctgtgagtgcattcttcttcttctttagtct
tcacagctaaactctggagagcttcaaaactagaaggatctactccgcatgggtg
catgcagaggctcctggatctgggaagcccgcctccacaaatgctgagccgt
tcttgctctgaaactgcgtgagtcaaggcaaatgcaaaaagccagggttttgggg
atgtgtcttactgtgcttcaacttcccaaggaattgaaagtcaacctaaactgta
acaacagggtgagaaatgaccaaactgcccgtagctttttctgaatggacttca
taaccggaagacttaaccgggtggcctcatcaccagagcatcgccaggatttcta
atgcactcagtttccctacatagcagggttcttagctaggtgtcccatgaac
cccgtaaagtctacacaaagtcttgcatacaggagcctttacaagatgattat
acagggttgacagattgggtgactgaccagacttggtgggggtcctgggatgagtt

- 8/9 -

gccccgggctgcaaattaagagtacagctaagtgcgggggtggcggtggagggga
acgaaaattgaacctgtctgcctgtgctgtgtcgtgtggctttatcagcccgag
gaagggcaggtgtattctaatttgcacaaaggtgctgggtagactagtggcagc
tctcatgtgctgcacataagtgggaatcagtatgaatagaagaacttgctgtata
aaggaatttcatggcaacaatgctggtaagggcaattagcctcgcttaagttgc
cttttttacacacacaaaactttttacatgaagggtggtttcacatgaatacta

FIG. 9 (continued)

tactgaaatctgtgccacaccaaactttttacatgaagggctgggtttcacatg
aatactatactgaaatctgtgctctcaagatctagcagtgaccagggctgcccg
gcgggggctctcctggcaagtcaggaagggtttctgttgctaataacatagaa
acacattagtgcactgggcctctctgaggtcagcatatttgtactcttggaata
tttggttttttcttcagtaacaacagaaaccccagttgggagtttaacaaataa
ctgactaccactcactcatgcattttttatttccaattaaagcaaagcactgtgc
tgtgctcagataataatagttttgtaagtaaaagtttttagttttcagtgttcag
gttatagaatataactgaccataaaaaattacctgcagggtattttctttttatga
acttggttttttaaattaccaagtaattactgggtgtcatttttggttttatgacaga
cacacgtatctaacaacaaacaaacagtgaccttctccatgggtcaaggactt
ccttacaatttctcctgagtttaacttttggtgaaaataatacctaagggttttctg
gcttattgaggaaatttcctaacaacaaacaaacaaacagaagagaaga
tcattaaccactgtatactttgtgtatataatagggtcagtgtaaagaaatatga
tttgagggtgggtgcatgcaagtaactagggtttatttctatataatgaatatattat
agatctgtaacatttggtttcaaaatgctgtttcattttttataaagtaccagtg
ttagctgctttttatacattaaattagcaatttgaaaaactc

- 1 -

SEQUENCE LISTING

<110> Bayer AG

<120> REGULATION OF HUMAN ADENYLATE CYCLASE, TYPE VII

<130> RCK-9 Foreign Countries

<140>

<141>

<150> US 60/254,782

<151> 2000-12-11

<160> 7

<170> PatentIn Ver. 2.1

<210> 1

<211> 3243

<212> DNA

<213> Homo sapiens

<400> 1

atgccagcca	aggggcgcta	cttctcgaac	gagggcgagg	agggccctga	ccaagatgcg	60
ctctacgaga	agtaccagct	caccagccag	catgggcccgc	tgctgtcac	gctcctgtcg	120
gtggccgcca	ctgcctgctg	ggccctcctc	atcattgcct	tcagccaggg	ggacccctcc	180
agacaccagg	ccattctggg	catggcgctt	ctgggtgctg	cgggtgttgc	ggccctctct	240
gtgctgatgt	acgtcgagtg	tctctcgagg	cgtggtctca	gggccttggc	gctgctcacc	300
tgggcctgct	tgggtggcgt	gggctatgtg	ctgggtgttc	acgcatggac	aaaggcggcc	360
tgtgcgtggg	agcaggtgoc	ottottcctg	ttcattgtct	tcgtgggtga	cacactactg	420
cccttcagca	tgcggggcgc	tgctgcggtt	ggggccgctc	ccactgcctc	ccacctcctg	480
gtgctcgggt	ctttgatggg	aggcttcacg	acaccagtg	tccgggtggg	gctgcagctg	540
ctggccaacg	cagtcattct	cctgtgtggg	aacctgacag	gcgccttcca	caagcaccaa	600
atgcaggatg	cgtcccggga	cctcttcacc	tacactgtga	agtgcattcca	gatccgcccg	660
aagctgcgca	tgcagaagcg	ccagcaggag	aacctgctgc	tgtcagtgct	tccggccccc	720
atctccatgg	gcattgaagc	ggccatcctc	gaacggctca	aggagcatgg	tgaccgtcgc	780
tgcatgcctg	acaacaactt	ccacagcctc	tacgtcaaga	ggcaccagaa	tgtcagcatc	840
ctctatcgcg	acatcgtggg	ottcagcgag	ctggccagcg	actgttctcc	caaggagctg	900
gtgggtggtg	tgaatgagct	ctttggcaag	ttcgaccaga	tcgccaaggc	caacgagtg	960
atgcgaatca	agatcctcgg	cgaactgctc	tactgtgtat	cgggcctgcc	cgtgtcgcctg	1020
cctacccacg	cccggaactg	cgtgaagatg	gggctggaca	tgtgccaggc	catcaagcag	1080
gtgcggggag	ccacggggct	ggacatcaac	atgcgtgtgg	gcatacactc	ggggaatgtg	1140
ctgtgcgggg	tcacggggct	gcgcaagtgg	cagtatgacg	tgtggtccca	cgaactgtcc	1200
ctggccaacc	ggatggaggc	agccggagta	cccgccggg	tgcacatcac	ggaggccacg	1260
ctaaagcacc	tggacaaggc	gtacgaggtg	gaggatgggc	acgggcagca	gcgggacccc	1320
tacctcaagg	agatgaacat	cgcacacctc	ctggtcatcg	acccccggag	ccagcagcca	1380
cccccgcccc	gccaacacct	ccccaggccc	aagggggacg	cggccctgaa	gatgcggggc	1440
tcagtgcgca	tgaccoggta	cctcgagtcc	tggggggcgg	cacggccctt	tgcacatctc	1500
aaccacogtg	agagcgtgag	cagtggtgag	acccacgtcc	ccaacggggc	gaggcctaag	1560
agcgtctccc	agcgccaccg	cgggacccca	cagacaagca	tgtcccccaa	ggggcggtcg	1620
gaggatgact	cgtacgatga	cagatgtctg	tcagccattg	aggggctcag	ctccacgagg	1680
ccctgtgtgt	ccaagtccga	tgaattctac	acctttgggt	ccattctcct	ggagaagggc	1740
tttgagcgcg	agtaaccgct	ggcaccctac	ccccggggcc	gccacgaatt	tgccctgcgc	1800
agcctgatct	tgtctgcat	cctgctcgtc	catgtcctgc	tcattgcccag	gacggcgcca	1860
ctgggtgtgt	ccttcgggct	ggtggcctgt	gtactggggc	tgggtgctgg	cctgtgattt	1920
gccaccaagt	tctcgaggtg	ctgccagctg	cgggggacgc	tctgcaactat	ctctgagagg	1980
gtggagagac	agccctgtct	gaggctgacc	ctggccgtcc	tgaecatcgg	cagcctgtct	2040

- 2 -

```

actgtggcca tcatcaacct gccctgatg cctttccaag ttccagagct gcctgttggc 2100
aatgagacag gcctactggc cgcgagcagc aagacaagag ccctgtgtga gcccctccc 2160
tactacacct gcagctgtgt cctgggcttc atcgctgtct cggctcttct gaggatgagc 2220
ctggagccaa aggttgtgtc gctgacagt ggcctgggtg cctacctggg gctcttcaac 2280
ctctcccat gctggcagt ggactgctgc ggccaaggcc tgggcaacct caccaagccc 2340
aacggcacca ccagtggcac ccctagctgt tcctggaagg acctgaagac catgaaccaat 2400
ttctacctgg tcctgttcta catcacctct cttacactct ccagacagat tgactattac 2460
tgccgcttgg actgcctatg gaagaagaag ttcaagaagg agcacgagga gtttgagacc 2520
atggagaacg tgaaccgcct tcttctggag aacgtcctgc cagcccacgt ggctgcccac 2580
tttatcggtg acaagttaaa cgaggactgg taccatcagt cctatgaact cgtctgtgtc 2640
atgtttgcct ccgtgcccga cttcaaatg ttctacacag agtgcgatgt caacaaagaa 2700
gggctggagt gcctacgcct gctcaatgag atcattgccg acttogacga gctcctactg 2760
aagcccaagt tcagcggcgt ggagaagatc aagaccatcg gcagcacgta catggcagct 2820
gcagggctca gcgtgcctc agggcacgag aaccaggagc tggagcggca gcatgcccac 2880
attggtgtca tgggtggagt cagcatcgcc ctgatgagta agctggacgg catcaacagg 2940
cactcettca actccttccg cctccgcgtc ggcataaacc atgggcctgt gattgctgga 3000
gtgattgggt cccgaaaacc tcagtatgac atctggggaa acactgtcaa tgtggccagc 3060
cgaatggaaa gcaactggaga acttgggaaa atccaggtta ccgaggagac ctgcaccatc 3120
ctccagggcc tcgggtactc ttgtgaatgc cgtggcctga tcaacgtcaa aggcaaaggc 3180
gagctgagga cttactttgt ctgtacggac actgccaagt ttcaggggct ggggctgaac 3240
tga 3243

```

<210> 2

<211> 1080

<212> PRT

<213> Homo sapiens

<400> 2

```

Met Pro Ala Lys Gly Arg Tyr Phe Leu Asn Glu Gly Glu Glu Gly Pro
  1              5              10              15

Asp Gln Asp Ala Leu Tyr Glu Lys Tyr Gln Leu Thr Ser Gln His Gly
      20              25              30

Pro Leu Leu Leu Thr Leu Leu Leu Val Ala Ala Thr Ala Cys Val Ala
      35              40              45

Leu Ile Ile Ile Ala Phe Ser Gln Gly Asp Pro Ser Arg His Gln Ala
      50              55              60

Ile Leu Gly Met Ala Phe Leu Val Leu Ala Val Phe Ala Ala Leu Ser
      65              70              75              80

Val Leu Met Tyr Val Glu Cys Leu Leu Arg Arg Trp Leu Arg Ala Leu
      85              90              95

Ala Leu Leu Thr Trp Ala Cys Leu Val Ala Leu Gly Tyr Val Leu Val
      100              105              110

Phe Asp Ala Trp Thr Lys Ala Ala Cys Ala Trp Glu Gln Val Pro Phe
      115              120              125

Phe Leu Phe Ile Val Phe Val Val Tyr Thr Leu Leu Pro Phe Ser Met
      130              135              140

Arg Gly Ala Val Ala Val Gly Ala Val Ser Thr Ala Ser His Leu Leu
      145              150              155              160

```

- 3 -

Val Leu Gly Ser Leu Met Gly Gly Phe Thr Thr Pro Ser Val Arg Val
 165 170 175
 Gly Leu Gln Leu Leu Ala Asn Ala Val Ile Phe Leu Cys Gly Asn Leu
 180 185 190
 Thr Gly Ala Phe His Lys His Gln Met Gln Asp Ala Ser Arg Asp Leu
 195 200 205
 Phe Thr Tyr Thr Val Lys Cys Ile Gln Ile Arg Arg Lys Leu Arg Ile
 210 215 220
 Glu Lys Arg Gln Gln Glu Asn Leu Leu Leu Ser Val Leu Pro Ala His
 225 230 235 240
 Ile Ser Met Gly Met Lys Leu Ala Ile Ile Glu Arg Leu Lys Glu His
 245 250 255
 Gly Asp Arg Arg Cys Met Pro Asp Asn Asn Phe His Ser Leu Tyr Val
 260 265 270
 Lys Arg His Gln Asn Val Ser Ile Leu Tyr Ala Asp Ile Val Gly Phe
 275 280 285
 Thr Gln Leu Ala Ser Asp Cys Ser Pro Lys Glu Leu Val Val Val Leu
 290 295 300
 Asn Glu Leu Phe Gly Lys Phe Asp Gln Ile Ala Lys Ala Asn Glu Cys
 305 310 315 320
 Met Arg Ile Lys Ile Leu Gly Asp Cys Tyr Tyr Cys Val Ser Gly Leu
 325 330 335
 Pro Val Ser Leu Pro Thr His Ala Arg Asn Cys Val Lys Met Gly Leu
 340 345 350
 Asp Met Cys Gln Ala Ile Lys Gln Val Arg Glu Ala Thr Gly Val Asp
 355 360 365
 Ile Asn Met Arg Val Gly Ile His Ser Gly Asn Val Leu Cys Gly Val
 370 375 380
 Ile Gly Leu Arg Lys Trp Gln Tyr Asp Val Trp Ser His Asp Val Ser
 385 390 395 400
 Leu Ala Asn Arg Met Glu Ala Ala Gly Val Pro Gly Arg Val His Ile
 405 410 415
 Thr Glu Ala Thr Leu Lys His Leu Asp Lys Ala Tyr Glu Val Glu Asp
 420 425 430
 Gly His Gly Gln Gln Arg Asp Pro Tyr Leu Lys Glu Met Asn Ile Arg
 435 440 445
 Thr Tyr Leu Val Ile Asp Pro Arg Ser Gln Gln Pro Pro Pro Pro Ser
 450 455 460
 Gln His Leu Pro Arg Pro Lys Gly Asp Ala Ala Leu Lys Met Arg Ala
 465 470 475 480

- 4 -

Ser Val Arg Met Thr Arg Tyr Leu Glu Ser Trp Gly Ala Ala Arg Pro
 485 490 495
 Phe Ala His Leu Asn His Arg Glu Ser Val Ser Ser Gly Glu Thr His
 500 505 510
 Val Pro Asn Gly Arg Arg Pro Lys Ser Val Pro Gln Arg His Arg Arg
 515 520 525
 Thr Pro Asp Arg Ser Met Ser Pro Lys Gly Arg Ser Glu Asp Asp Ser
 530 535 540
 Tyr Asp Asp Glu Met Leu Ser Ala Ile Glu Gly Leu Ser Ser Thr Arg
 545 550 555 560
 Pro Cys Cys Ser Lys Ser Asp Asp Phe Tyr Thr Phe Gly Ser Ile Phe
 565 570 575
 Leu Glu Lys Gly Phe Glu Arg Glu Tyr Arg Leu Ala Pro Ile Pro Arg
 580 585 590
 Ala Arg His Asp Phe Ala Cys Ala Ser Leu Ile Phe Val Cys Ile Leu
 595 600 605
 Leu Val His Val Leu Leu Met Pro Arg Thr Ala Ala Leu Gly Val Ser
 610 615 620
 Phe Gly Leu Val Ala Cys Val Leu Gly Leu Val Leu Gly Leu Cys Phe
 625 630 635 640
 Ala Thr Lys Phe Ser Arg Cys Cys Pro Ala Arg Gly Thr Leu Cys Thr
 645 650 655
 Ile Ser Glu Arg Val Glu Thr Gln Pro Leu Leu Arg Leu Thr Leu Ala
 660 665 670
 Val Leu Thr Ile Gly Ser Leu Leu Thr Val Ala Ile Ile Asn Leu Pro
 675 680 685
 Leu Met Pro Phe Gln Val Pro Glu Leu Pro Val Gly Asn Glu Thr Gly
 690 695 700
 Leu Leu Ala Ala Ser Ser Lys Thr Arg Ala Leu Cys Glu Pro Leu Pro
 705 710 715 720
 Tyr Tyr Thr Cys Ser Cys Val Leu Gly Phe Ile Ala Cys Ser Val Phe
 725 730 735
 Leu Arg Met Ser Leu Glu Pro Lys Val Val Leu Leu Thr Val Ala Leu
 740 745 750
 Val Ala Tyr Leu Val Leu Phe Asn Leu Ser Pro Cys Trp Gln Trp Asp
 755 760 765
 Cys Cys Gly Gln Gly Leu Gly Asn Leu Thr Lys Pro Asn Gly Thr Thr
 770 775 780

- 5 -

Ser Gly Thr Pro Ser Cys Ser Trp Lys Asp Leu Lys Thr Met Thr Asn
 785 790 795 800
 Phe Tyr Leu Val Leu Phe Tyr Ile Thr Leu Leu Thr Leu Ser Arg Gln
 805 810 815
 Ile Asp Tyr Tyr Cys Arg Leu Asp Cys Leu Trp Lys Lys Lys Phe Lys
 820 825 830
 Lys Glu His Glu Glu Phe Glu Thr Met Glu Asn Val Asn Arg Leu Leu
 835 840 845
 Leu Glu Asn Val Leu Pro Ala His Val Ala Ala His Phe Ile Gly Asp
 850 855 860
 Lys Leu Asn Glu Asp Trp Tyr His Gln Ser Tyr Asp Cys Val Cys Val
 865 870 875 880
 Met Phe Ala Ser Val Pro Asp Phe Lys Val Phe Tyr Thr Glu Cys Asp
 885 890 895
 Val Asn Lys Glu Gly Leu Glu Cys Leu Arg Leu Leu Asn Glu Ile Ile
 900 905 910
 Ala Asp Phe Asp Glu Leu Leu Leu Lys Pro Lys Phe Ser Gly Val Glu
 915 920 925
 Lys Ile Lys Thr Ile Gly Ser Thr Tyr Met Ala Ala Ala Gly Leu Ser
 930 935 940
 Val Ala Ser Gly His Glu Asn Gln Glu Leu Glu Arg Gln His Ala His
 945 950 955 960
 Ile Gly Val Met Val Glu Phe Ser Ile Ala Leu Met Ser Lys Leu Asp
 965 970 975
 Gly Ile Asn Arg His Ser Phe Asn Ser Phe Arg Leu Arg Val Gly Ile
 980 985 990
 Asn His Gly Pro Val Ile Ala Gly Val Ile Gly Ala Arg Lys Pro Gln
 995 1000 1005
 Tyr Asp Ile Trp Gly Asn Thr Val Asn Val Ala Ser Arg Met Glu Ser
 1010 1015 1020
 Thr Gly Glu Leu Gly Lys Ile Gln Val Thr Glu Glu Thr Cys Thr Ile
 1025 1030 1035 1040
 Leu Gln Gly Leu Gly Tyr Ser Cys Glu Cys Arg Gly Leu Ile Asn Val
 1045 1050 1055
 Lys Gly Lys Gly Glu Leu Arg Thr Tyr Phe Val Cys Thr Asp Thr Ala
 1060 1065 1070
 Lys Phe Gln Gly Leu Gly Leu Asn
 1075 1080

- 6 -

<210> 3
<211> 22
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)..(22)
<223> Primer1

<400> 3
ccggacccca gacagaagca tg

22

<210> 4
<211> 23
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)..(23)
<223> Primer 2

<400> 4
ggatgcagac gaagatcagg ctg

23

<210> 5
<211> 23
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)..(23)
<223> Primer:AC7-L

<400> 5
tcagcgtcgc ctcagggcac gag

23

<210> 6
<211> 22
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)..(22)
<223> Primer:AC7-R

<400> 6
gaggccctgg aggatggtgc ag

22

<210> 7
<211> 6196
<212> DNA

- 7 -

<213> Homo sapiens

<400> 7

tgagggaactg	cgtgtggagt	cagcccagtc	tggatgcaca	ggaggatgct	ggcggcacag	60
tgagtgaggo	ctgggtgccag	agctgtgcgg	accccttgtt	ggccatggag	cagcaggccc	120
agaggccctc	tccccagccc	tgtttgcctg	cctcggagag	gacagaggcc	taggcccacg	180
ggggagggtg	ttggcagaca	gatgccctcc	aggccctggg	gcctccttaa	cgccccctta	240
acgacacgcg	tgccaagggt	ggaggatgcc	agccaagggg	cgctacttcc	tcaacgaggg	300
cgaggagggc	cctgaccaag	atgcgctcta	cgagaagtac	cagctcaoca	gccagcatgg	360
gccgtctcg	ctcacggctcc	tgctgggtgg	cgccaactgoc	tgctggccc	tcatcatcat	420
tgcccttcagc	caggggggacc	cctccagaca	ccaggccatt	ctgggcatgg	cgttccctgg	480
gctggcggtg	tttgcgcccc	tctctgtgct	gatgtacgtc	gagtgtctcc	tgcgcgctg	540
gctcagggcc	ttggcgctgc	tcacctgggc	ctgcttgggtg	gcgctgggct	atgtgtctgg	600
gttcgacgca	tgacaaaagg	cgccctgtgc	gtggggagcag	gtgcccttct	tcctgttcat	660
tgtcttcgtg	gtgtacacac	tactgccctt	cagcatgogg	ggcgctgtcg	cogttggggc	720
cgtctccact	gcctcccacc	tcctgggtgt	cggttctttg	atgggaggct	tcaogacaoc	780
cagctgctcg	atcacggctgc	agctgggtgg	caacgcagtc	atcttctgtg	gtgggaacct	840
gacaggcgcc	ttccacaagg	accaaattgca	ggatgcgtcc	cgggacctct	tcaoctacac	900
tgtgaagtgc	atccagatcc	gcccgaagct	gcgcctogag	aagcgccagc	aggagaacct	960
gctgctgtca	gtgcttccgg	cccacatctc	catgggcatg	aagctggcca	tcatcgaaag	1020
gctcaaggag	catggtgacc	gtcgctgcat	gcctgacaac	aacttccaca	gcctctacgt	1080
caagaggcac	cagaatgtca	gcctcctcta	tgccgacatc	gtgggcttca	cgcagctggc	1140
cagcgactgt	tctcccaagg	agctgggtgg	ggtgctgaat	gagctctttg	gcaagttcga	1200
ccagatcgcc	aaggccaacg	agtgcctgct	aatcaagatc	ctcgccgact	gctactactg	1260
tgtatcgggc	ctgcccgtgt	cgctgcctac	ccacgcccgg	aactgcgtga	agatggggct	1320
ggacatgtgc	caggccatca	agcagggtgc	ggaggccacg	ggcgctggaca	tcaacatgcg	1380
tgtgggcata	cactcgggga	atgtgctgtg	cggggtcatc	gggctgcgca	agtggcagta	1440
tgacgtgtgg	tcccacgacg	tgctccctgg	caaccggatg	gaggcagccg	gagtaccocg	1500
ccgcgtcgac	ccacgctaac	ccacgctaac	aaggcgtagc	aaggcgtagc	aggtggagga	1560
tgggcaocgg	cagcagcggg	acccctacct	caaggagatg	aacatccgca	cctacctggg	1620
catcgacccc	cggagccagc	agccaccccc	gcccagccaa	cacctcccca	ggcccaaggg	1680
ggacgcggcc	ctgaagatgc	ggcgctcagt	gcgcctgacc	cggtacctcg	agtcctgggg	1740
ggcggcacgg	ccctttgcac	atctcaacaa	cogtgagagc	gtgagcagtg	gtgagacca	1800
cgtccccaac	ggcgggaggc	ctaagagcgt	tccccagcgc	caccgcccga	cccagacag	1860
aagcatctcc	cccaaggggc	ggtcgaggga	tgactcgtac	gatgacgaga	tgctgtcagc	1920
cattgagggg	ctcagctcca	cgaggccctg	ctgctccaag	tcgatgact	tctacacctt	1980
tggttccatc	ttcctggaga	agggctttga	gcgcaggtac	cgctgggac	ccatcccccg	2040
ggcccgccac	gactttgcct	gcgcagcct	gatcttcgtc	tgcatcctgc	tcgtccatgt	2100
cctgctcatg	cccaggacgg	cggaactggg	tgtgtccttc	gggctgggtg	cctgtgtact	2160
ggggtctgtg	ctgggocctg	gctttgccac	caagtctctg	aggtgctgcc	cagctcgggg	2220
gacgtctctg	actatctctg	agagggtgga	gacacagccc	ctgctgaggc	tgacctggc	2280
cgtcctgacc	atcggcagcc	tgctcactgt	ggccatcatc	aacctgcccc	tgatgccttt	2340
ccaagttcca	gagctgocct	ttggcaatga	gacaggccta	ctggccgcga	gcagcaagac	2400
aagagccctg	tgtgagcccc	tcccgactta	cacctgcagc	tgtgtcctgg	gcttcatcgc	2460
ctgctcggtc	ttcctgagga	tgagcctgga	gccaagggtt	gtgctgctga	cagtggccct	2520
ggtggccctac	ctggtgctct	tcaacctctc	ccatgctgg	cagtgggact	gctgcccga	2580
aggcctgggc	aacctcacca	agcccaacgg	caccaccagt	ggcacccta	gctgttctg	2640
gaaggacatg	aagaccatga	ccaatttcta	cctggctcct	ttctacatca	ccctgcttac	2700
actctccaga	cagattgact	attactgcgc	cttggaactg	ctatggaaga	agaagttcaa	2760
gaaggagcac	gaggagtttg	agaccatgga	gaacgtgaac	cgccttcttc	tgagaaacgt	2820
cctgccagcc	cacgtggctg	cccactttat	cggtgacaag	ttaaacgagg	actggtacca	2880
tcagtcctat	gactgogtct	gtgtcatggt	tgccctcctg	ccggacttca	aagtgttota	2940
cacagagtgc	gatgtcaaca	aagaagggtt	ggagtgccta	cgcctgctca	atgatcatat	3000
tgccgacttc	gacgagctcc	tactgaagcc	caagttcagc	ggcggtggga	agatcaagac	3060
catcggcagc	acgtacatgg	cagctgcagg	gctcagcgtc	gcctcagggc	acgagaacca	3120
ggagctggag	cggcagcatg	cccacattgg	tgtcatgggtg	gagttcagca	tcgccctgat	3180
gagtaagctg	gacggcatca	acaggcactc	cttcaactcc	ttccgcctcc	gcgtcggcat	3240
aaaccatggg	cctgtgattg	ctggagtgat	tggggcccca	aaacctcagt	atgacatctg	3300
gggaacaact	gtcaatgtgg	ccagccgaat	ggaaagcact	ggagaacttg	ggaaactcca	3360

ggttaccgag	gagacctgca	ccatcctcca	gggcctcggg	tactcttgtg	aatgcogtgg	3420
cctgatcaac	gtcaaaggca	aaggcgagct	gaggacttac	tttgtctgta	cggacactgc	3480
caagtttcag	gggctggggc	tgaactgagg	gctcctgctg	gattccgaaa	aggccgggaa	3540
gccagtctcc	ttccctgaag	caagcccagg	agaagactct	ccgccccacg	ccaatcccaa	3600
aggcatgcag	atggctgtgc	atgttggctt	ctttggacct	gcactggagg	atttctcaga	3660
cacatgcacc	agattctggc	tcgaagcagc	cactgagcca	taatgcgcag	gggaggccag	3720
aagctctgtg	cctggctctgt	aacagtttcc	aggccagctg	gagaatgttc	actggttcgg	3780
ggctgacttt	gagatctttg	ttccctgagg	tgccaggcag	gcaactttag	cacatgatga	3840
aaacagactt	ccacctcagt	ggcctgtggg	cacgcacaag	tgaggctctgt	ttttctagac	3900
accaaggggg	agtaagctga	gctgtctagc	acggatggga	gactccctct	ccctgggtggg	3960
cctggcaatg	acagcatttc	tcacagaggg	attctggtaa	atgaagctga	aagggggtgtt	4020
ttacatctgt	aaacggtttc	aaacaggtag	agagaaaaac	accacaatta	acactgtttac	4080
ttttttgcott	gtctggcatg	tttgttttaa	atgaatacat	taatgggggtt	tttatcctttt	4140
tgaatgactt	ttcagacact	agacataaat	ctcttccctc	cagtgtatgc	tctgcctttt	4200
taaccactga	catgtaagga	ggactactgt	ctagcatcag	cttatgggggt	cagctggctg	4260
tggggataga	gtcctgagga	atgtggtcac	agcaagaagg	cggggagcag	cagagccttg	4320
cctttgaatg	aggcagcttg	tgaggcaagc	attctggaga	gagggtgottt	gaaagtaagg	4380
tgccggccttt	cacctcttcc	ttgattactc	acacatcttt	gcgttctccc	ctgcgcgtcct	4440
tcaactgtat	cttacttttc	ttaccagaaa	ggaatggagt	ctgttttagag	acaacttgga	4500
caacctgtga	gtgcatctct	tctttccttt	agtcttcaca	gctaactctg	gagagcttca	4560
aaactagaag	gatctactcc	gcatgggtgc	atgcagaggc	tcctggatct	gggaagcccg	4620
ccccctcaca	aatgctgagc	cgttcttgc	ctgaaactgc	gtgagtcaag	gcaaatgcaa	4680
aaagccaggt	tttggggatg	tgtcttactg	tgtctcaact	tcccaaggaa	ttgaaagtca	4740
acctaactgt	aacaacaggg	tgagaaatga	ccaaactgcc	cgtgactttt	tctgaaatgga	4800
cttcataacc	ggaagactta	accggtggcc	tcatcaccag	agcatcgcca	ggattttctaa	4860
tgactcagct	ttccctacat	agcagggatt	cttagctagg	tgtcccatg	aaccccgtaa	4920
agttctacac	aaagtcttgc	atacaggagc	ctttacaaga	tgattataca	gggtttgcaga	4980
ttgggtgact	gaccagactt	gttgggggtcc	tgggatgagt	tgccccgggc	tgcaaattaa	5040
gagtacagct	aagtgcgggg	gtggcgggtg	agggaaogaa	aattgaacct	gtctgcctgt	5100
gctgtgtcgt	gtggccttat	cagcccgagg	aagggcaggt	gtattctaat	ttgcacaaaag	5160
gtgctgggta	gactagtggc	agctctcatg	tgctgcacat	aagtggaato	agtatgaata	5220
gaagaacttg	ctgtataaag	gaatttcatg	gcaacaatgc	tggttaagggc	aattagcctc	5280
gcttaagttg	cctttttttac	acacccaaaac	ttttttacatg	aagggctgggt	ttcacatgaa	5340
tactatactg	aaatctgtgc	cacacccaaaa	ctttttacat	gaagggctgg	tttcacatga	5400
atactatact	gaaatctgtg	ctctcaagat	ctagcagtga	ccagggctgc	ccggcggggg	5460
ctctcctggc	aagtcaggaa	ggtttctgtt	gctaatataa	catagaaaaca	cattagtgc	5520
ctgggcctct	ctgaggtcag	catatttcta	ctcttgggaat	atttgttttt	ttotttcagta	5580
acaacagaaa	ccccagttgg	gagtttaaca	aataactgac	taccaactcac	tcattgcattt	5640
ttattttccaa	ttaaagcaaa	gcactgtgct	gtgctcagat	aataatagtt	tgtaagtata	5700
agtttttagt	tttcagtgtt	cagggttatag	aataataactg	accataaaaa	ttacotgcag	5760
gtattttctt	tttatgaact	tgtttttaaa	ttaccaagta	attactgggtg	tcattttgtt	5820
ttatgacaga	cacacgtatc	taacaaaaca	acaaacagtg	acottctcca	tgggtcaagg	5880
acttccttac	aatttctcct	gagtttaact	ttgtgaaaaat	aatacctaag	gttttctggc	5940
ttattgagga	aatttctctaa	caaacaaaaca	aacaaacaaa	cagaagagaa	gatcattaac	6000
cactgtatac	tttgtgtata	taatagggtca	gtgtaaagaa	atatgatttg	aggtgggtgca	6060
tgcaagtaac	tagggtttat	tctatataat	gaatatttat	agatctgtaa	catttgtttc	6120
aaaatgctgt	ttcattttta	taaagtacca	gtgttttagct	gottttttata	cattaaatta	6180
gcaatttgaa	aaactc					6196

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
20 June 2002 (20.06.2002)

PCT

(10) International Publication Number
WO 2002/048397 A3

(51) International Patent Classification⁷: **C12Q 1/68**,
C12N 9/88, A61K 39/00, 48/00, A61P 11/06, 29/00,
31/00, 37/08, G01N 33/53

(21) International Application Number:
PCT/EP2001/014398

(22) International Filing Date: 7 December 2001 (07.12.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/254,782 11 December 2000 (11.12.2000) US

(71) Applicant (for all designated States except US): **BAYER AKTIENGESELLSCHAFT** [DE/DE]; 51368 Leverkusen (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **LIU, Ningshu** [CN/JP]; 4-39-305, Sanjomiyamae-cho, Nara-shi, Nara 630-8121 (JP). **MANABE, Yoshihisa** [JP/JP]; 4-8-19, Suzaku, Nara-shi, Nara 631-0806 (JP).

(74) Common Representative: **BAYER AKTIENGESELLSCHAFT**; 51368 Leverkusen (DE).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:
8 January 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: REGULATION OF HUMAN ADENYLATE CYCLASE, TYPE VII

(57) Abstract: An adenylate cyclase type VII protein, cDNA, and reagents which regulate human adenylate cyclase type VII can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, diseases that are caused by aberrant activity of this enzyme and diseases whose symptoms can be ameliorated by stimulating or inhibiting the activity of type VII adenylate cyclase. Such diseases include, for example, diseases caused by allergic or inflammatory reaction, such as asthma, allergic rhinitis, atopic dermatitis, food allergy, contact allergy, hives conjunctivitis, vernal catarrh; autoimmune diseases, COPD, inflammatory bowel diseases, rheumatoid arthritis, osteoarthritis, sepsis/septic shock, systemic inflammatory response syndrome, polymyositis, dermatomyositis, polyarthritis nodosa, mixed connective tissue disease, Sjogren's syndrome, gout, and bacterial, fungal, and viral infections, among others.

WO 2002/048397 A3

INTERNATIONAL SEARCH REPORT

Application No
PCT/EP 01/14398

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68 C12N9/88 A61K39/00 A61K48/00 A61P11/06
A61P29/00 A61P31/00 A61P37/08 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 107 076 A (GILMAN ALFRED G ET AL) 22 August 2000 (2000-08-22) column 1, line 27 - line 29 column 17, line 25 - column 18, line 23	1-4, 9-42
X	WATSON P.A. ET AL.: "Molecular Cloning and Characterization of Type VII Isoform of Mammalian Adenylyl Cyclase Expressed Widely in Mouse Tissues and in S49 Mouse Lymphoma Cells" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 46, 18 November 1994 (1994-11-18), pages 28893-28898, XP002227421 page 28893, column 2, line 1 - line 4; figures 1,3,5	1-4, 9-42

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *8* document member of the same patent family

Date of the actual completion of the international search

20 January 2003

Date of mailing of the international search report

04/02/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Schönwasser, D

INTERNATIONAL SEARCH REPORT

Inter Application No

PCT/EP 01/14398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEITZ E. ET AL.: "Adenylyl Cyclase Type 7 Is the Predominant Isoform in the Bovine Retinal Pigment Epithelium" ACTA ANATOMICA, vol. 162, no. 2-3, 1998, pages 157-162, XP008012572 page 159, column 1, paragraph 3 -column 2, paragraph 1 page 160, column 1, line 1 - line 5 ----	1-4,9-32
A	VÖLKEL H. ET AL.: "Cloning and expression of a bovine adenylyl cyclase type VII specific to the retinal pigment epithelium" FEBS LETTERS, vol. 378, 1996, pages 245-249, XP002227422 figures 2,3 ----	1-4,9-42
A	NOMURA N ET AL: "PREDICTION OF THE CODING SEQUENCES OF UNIDENTIFIED HUMAN GENES. I. THE CODING SEQUENCES OF 40 NEW GENES (KIA A0001-KIA A0040) DEDUCED BY ANALYSIS OF RANDOMLY SAMPLED CDNA CLONES FROM HUMAN IMMATURE MYELOID CELL LINE KG-1" DNA RESEARCH, vol. 1, no. 1, 1994, pages 27-35, XP002913067 ISSN: 1340-2838 KIAA0037 - Table 4, 4th gene number from the bottom of the table ----	1-4,9-42
A	DEFER N. ET AL.: "Tissue specificity and physiological relevance of various isoforms of adenylyl cyclase" AMERICAN JOURNAL OF PHYSIOLOGY, vol. 279, September 2000 (2000-09), pages F400-F416, XP002227423 the whole document ----	1-4,9-42
A	WO 97/40170 A (CADUS PHARMACEUTICAL CORP) 30 October 1997 (1997-10-30) page 43, line 1 -page 71, line 34 page 83, line 25 -page 91, line 7 -----	1-4,9-42

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

THIS PAGE BLANK (USPTO)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 5-8

Present claims 5-8, 25, 26, 33 and 35 relate to a products or methods defined by reference to a desirable characteristic or property, namely a reagent that modulates the activity of or binds to a defined polynucleotide or polypeptide (here: human adenylate cyclase type VII). The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the products by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts of claims 25, 26, 33 and 35 relating products or methods involving hAC7-specific antibodies, RNA molecules, antisense oligonucleotides and ribozyme as described in on page 26, line 29 to page 33, line 30.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.